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Isolation and identification of Pathogenic Bacteria from *Cyprinus carpio* L. by Vitek 2 System

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Abstract

Fish are vulnerable to a wide range of bacterial infections that are naturally occurring in the surroundings. The current study's goal was to isolate the pathogenic bacteria from common carp sold in ponds at the city of Baqubah which utilize as most popular fish mail and principle food. Sixty fish samples were obtained from five places in Baqubah city (12 samples from AL-Mafraq, 12 samples from AL-Razi, 12 samples from AL-Moalemmen District , 12 samples from AL-Rahma and 12 samples from Old Baqubah). The samples were taken from fish from each location. Anatomical process to the each fish was done and then bacterial strains were isolated from several parts of fish body (skin, gills ,liver and intestine). All samples were cultivated on blood agar medium as an enriched medium and MacConkey agar as a differentiated medium after collection, and they were both hatched on 37 ° C for 24 hr. The colony morphology, microscopic analysis, and biochemical assays were used to identify probable isolates. , then confirmed and sensitivity were done by VITEK 2 system. The bacterial isolated from each target organs showed that the potential pathogenic bacteria which establish in carp from this investigation w*ere E.coli* 30%,*Klebseilla spp* 30% ,*Pseudomonas spp* 30% *and salmonella spp* 10% *i*n addition to other species such as *Staphylococcus spp, Proteus spp* and others. Sensitivity test that performed by Vetk2 system showed high rates of resistance to the wide spectrum antibiotics which commercially used in Iraq. Fish handling or preparation errors may put consumers' food safety at risk because of the existence of pathogenic bacteria in the fishes.

Key words : Bacteria, Common carp , vitek

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Introduction

In aquaculture, bacterial infections cause significant economic losses. Stress caused by seasonal fluctuations in water quality and stressful stock circumstances leads common carp grown for food to become repeatedly infected by opportunistic infections. [1]. Common carp , grass carp and silver carp are the three maximum prevalent fish species found in fish farms in Iraq. (2) . Fish farms are impacted by a variety of pathogenic microorganisms, including bacteria, viruses, parasites, and protozoa. In addition, these farms may also be afflicted by non-infectious diseases. (3). . Fish meat is one of the most significant feed ingredients since it is one of the least expensive sources of animal protein in recent years. It also has good nutritional value because it is rich in proteins, vitamins, and unsaturated fatty acids. (4). Fish become polluted due to their unfavorable environment, including manure, polluted water, the harvesting region, and from pollution by employees, instruments, apparatus, and unclean management, which results in the presence of a large number of bacteria in the fish. (5), Numerous studies on the bacterial ecology of fish have been conducted in latest years. Some of these studies show that the epidermis and interior organs of vigorous, newly caught fish are microbiologically sterilized, nevertheless other studies have establish the occurrence of bacteria in fish muscles. (5). Fish is a healthy diet for people, and the flesh of fish is full of nutrients, minerals, and fat. It provides roughly sixteen percent of the animal protein consumed by people worldwide , making it the greatest significant foundation of excellent protein for humans., however, The fish are sensitive to a number of bacterial infections, some of which are thought to be saprophytic in nature and are able to cause disease. Fresh fish muscle's microbial richness is influenced by the surrounding environment and fishing areas. It has been hypothesized that a fish's habitat influences the sort of microorganisms that are present in association with it. There are two types of bacterial infections linked to fish: indigenous and non-indigenous. Although many of the coliforms are harmless to people, their presence suggests that other microbes may be hazardous. (6). among the numerous hundreds of bacterial or archaeal kinds found in animal-associated microflora are considered to influence host health, behavior, and development. (7). Alternatively, microbes, living in a diversity

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of positions through the animal body, comprising relations through non-gastrointestinal mucosal surfaces, which besides contribute in biological or gas conversation in addition to barricades or entrance topics for pathogens. Therefore, it is crucial to characterize non-gastrointestinal mucosal microbiomes, like those of the gills and skin in order to spot new behaviors of animal-related microbial flora and comprehend the relationship among both the host and environmental microbial populations . The study aimed to detect the presence and spread of *bacterial isolate* in fish available on farms and determine the resistance to antibiotics of these isolated.

Materials and methods

Sampling

Sixty fish samples (*Cyprinus carpio*) were collected in the current study and were obtained from five places in Baqubah city (AL-Mafraq, AL-Razi, AL-Moalemmen District ,AL-Rahma and Old Baqubah) at the period from November 2021 to January, 2022.

Isolation of bacteria

The samples were directly transported to the Microbiology - Veterinary Medicine / Diyala University laboratory, in antiseptic circumstances, Fish was aseptically dissected, and pieces of the skin, gills, intestines, and liver were hatched in a tube of ten milliliters of the brain-heart infusion broth aimed at 24 hr. in an aerobic culture at 37 °C. Fish surface cotton swaps were then added to the tube. A loopful of the incubated broth was streaked at 37°C for 24 hours on blood agar, MacConkey, mannitol salt agar and SS agar incubation plates for microbiological analysis.

Identification of bacteria

Following isolation, Gram's staining, phenotypic traits (colonial morphology, microscopic appearance), and biochemical tests were carried out to identify the bacteria using the identification keys that were made available in the studies by Mondal (8)

The isolates' pure cultures were known by biochemical characterization consuming the principles defined in Bergey's Manual of Formative Bacteriology (9). Also , VITEK 2 system was used to confirm and determine the sensitivity test for each strain .

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Results and discussion

Signs and Symptoms of fish infection

Some signs and symptoms fish specimens were gathered from the five places in Baqubah city were saw, including unusual behavior, reddish tints or patches on the eyes, gills, skin, or fins, bloated abdomens, hemorrhage of the skin and interior organs, and swelling eyes.

Isolation and documentation of bacteria

The result of current study showed that out of 60 samples (*E.coli 30%,Klebseilla spp30% ,Pseudomonas spp 30% and salmonella spp10%*). isolated and recognized from the farms in were obtained from five places in Baqubah city (AL-Mafraq,AL-Razi, AL-Moalemmen District ,AL-Rahma and Old Baqubah) are dangerous and could have a negative impact on public health if used. (Table 1 + 2).

For the separation of bacterial species' pure colonies, numerous kinds of selective, enriched, and differential medium were utilized . For the screening of G- bacteria in contrast to Gram + bacteria, crystal violet and methyl red markers were used, on several types of medium, growth was seen in all isolated species. The isolation and characterization of four bacterial species, such as(*E.coli , Klebseilla Pseudomonas and Salmonella*) Confirmatory diagnosis the results of the VITEK 2 technique showed that the isolate had a 100 % identity with a probability of 99%.

Table 1. bacterial spp. isolated from common carp's skin, gills, intestines, and liver

Bacteria	Skin	Gills	Intestine	Liver
E. coli	+	+	+	+
Klebsiella	+	+	-	-
Pseudomonas	+	+	+	-
Salmonella	+	-	+	-

(+): present , (-): not present

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Table 2. percentage (%) of bacteria that were isolated from public carp's skin, gills, liver, and intestines.

Bacteria	Skin	Gills	Intestine	Liver	Total
E.coli	10%	5%	9%	6%	30%
Klebsiella	17%	13%	-	-	30%
Pseudomonas	7%	14%	9%	-	30%
Salmonella	4%	-	6%	-	10%

The most effective antibiotics' susceptibility were meropenem and Imipenem(<= 0. 25 µg/ml) and ertapenem (<= 0. 5 µg/ml) when examined on *E.coli* and *klebsiella* whereas, it was unaffected to Trimethoprim/Sulfa methoxazole (<=320 µg/ml) (Table 3 and 4) :

Table 3: Antibiotic susceptibility of *E.coli*

Antibiotics	MIC	Interpretation	Antibiotics	MIC	Interpretation
Ampicillin	>=32	R	Meropenem	<= 0. 25	S
Amoxicillin/clavulanic	8	S	Amikacin	<= 2	S
Piperacillin/ Tazobactan	<= 4	S	Gentamicin	>=16	R
Cefotaxime	>=64	R	Ciprofloxacin	>=4	R
Ceftazidime	16	R	Norfloxacin	>=16	R
Cefepime	8	R	Fosfomycin	<= 16	S
Ertapenem	<= 0. 5	S	Nitrofurantion	<= 16	S
Imipenem	<= 0. 25	S	Trimethoprim/Sulfa methoxazole	>=320	R

*MIC: Minimum Inhibitory Concentration (µg/ml), R: Resistant, S: Sensitive,

Table 4: Antibiotic susceptibility of *klebsiella*

Antibiotics	MIC	Interpretation	Antibiotics	MIC	Interpretation
Ampicillin	>=32	R	Meropenem	<= 0. 25	S
Amoxicillin/clavulanic	16	S	Amikacin	<= 2	S
Piperacillin/ Tazobactan	8	S	Gentamicin	<=1	S
Cefotaxime	>=64	R	Ciprofloxacin	1	S
Ceftazidime	4	R	Norfloxacin	2	S
Cefepime	2	R	Fosfomycin	<= 16	S
Ertapenem	<= 0. 5	S	Nitrofurantion	64	I
Imipenem	<= 0. 25	S	Trimethoprim/Sulfa methoxazole	>=320	R

The more antibiotic susceptibility to pseudomonas aeruginosa were Meropenem and Ciprofloxacin (<= 0. 25) while , it was resistant to Cefotaxime (>=64).and The greatest

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antibiotics susceptibility were meropenem and Imipenem(<= 0. 25 µg/ml) and ertapenem (<= 0. 5 µg/ml) when tested on *salmonella typhi* while, it was resistant to Cefotaxime ,Ceftazidime and Cefepime table (6).

Table 5: Antibiotic susceptibility of *pseudomonas aeruginosa*

Antibiotics	MIC	Interpretation	Antibiotics	MIC	Interpretation
Ampicillin			Meropenem	<= 0. 25	S
Amoxicillin/clavulanic			Amikacin	<= 2	S
Piperacillin/ Tazobactan	16	S	Gentamicin	2	s
Cefotaxime	>=64	R	Ciprofloxacin	<=0.25	s
Ceftazidime	4	s	Norfloxacin	1	s
Cefepime	4	s	Fosfomycin		
Ertapenem			Nitrofurantion		
Imipenem	2	S	Trimethoprim/Sulfa methoxazole		

Table 6: Antibiotic susceptibility of *salmonella typhi*

Antibiotics	MIC	Interpretation	Antibiotics	MIC	Interpretation
Ampicillin	>=32	R	Meropenem	<= 0. 25	S
Amoxicillin/clavulanic	4	S	Amikacin	<= 2	R
Piperacillin/ Tazobactan	<=4	S	Gentamicin	<=1	R
Cefotaxime	>=64	R	Ciprofloxacin	0.5	s
Ceftazidime	>=64	R	Norfloxacin	2	s
Cefepime	>=64	R	Fosfomycin	<=16	S
Ertapenem	<=0.5	S	Nitrofurantion	32	S
Imipenem	<=0.25	S	Trimethoprim/Sulfa methoxazole	<=20	S

The aquatic ecosystem contains a substantial amount of microflora, and the interaction of this aquatic environment with pathogenic microflora leads to infectious diseases in aquaculture techniques. (10). Aquatic water bodies become contaminated due to the presence of pathogenic bacterial species that are brought by animal feces. (11). The discovery of ***E****scherichia coli, Shigella, and Salmonella* in the fishes suggests that the environment has been contaminated by feces. (12).

Four bacteria species from the skin, gills, intestine and liver were found in this

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investigation. This outcome is consistent with the findings of (13), who found six different bacterial species on the skin, gills, and buccal cavity of *S. esocinus*. Six bacterial species, including *Staphylococcus., Enterobacter., Pseudomonas, Bacillus,., E. coli, and Salmonella.*, were found among the isolated microorganisms.

The existence of *Salmonella and Bacillus* species are too significant, since they may effect in food borne infections. *Salmonella* sp. is a sign of improper food handling procedures, including cross contamination.. Greatest of the isolated organisms are possibly pollutants, especially afterward the smoking procedure, and they are frequently decay organisms.(13)

Fish that have enteric bacteria in them are a good indicator of contaminated feces and polluted water. Given the presence of pathogenic bacteria happening fish, it was proposed that improper handling or preparation of fish could put consumer food safety at risk. (6). The microorganisms for example *Pseudomonas , Staph, Salmonella and shigella* are existing in nutrition substantial, producing greatest foodborne illnesses, as the situation besides customers.

In similar studies, *E .coli, Pseudomonas, S. aureus, S .typhi, and S. dysenteriae* were isolated from the skin, intestines, gills, and muscles of *Megalaspis cordyla* and *Pricanthus hamrur* muscles in royapuram water in India. .[14]

It is possible to perform a drug inspection process with high sensitivity in a variety of organisms, which can clearly reduce the prescription of antibiotics, on the opposite hand, The use of antibiotics carries a number of risks., initially, the medication can enter live tissues and membranes ,like , it can pass through brain blood vessels , additionally, use antibiotics for an extended period of time results in bacterial resistance, drug accumulation in fish tissues, and ecological difficulties .

We can draw the conclusion that fish are susceptible to any contaminants that might be in the water. After the harvest , handling, distributing, and sale of fish, These treatments will cause microbial activities that will lower the quality of the fish meat., Thus, in order to safeguard customers against the risk to the public's health, the fundamental guidelines for the prevention of food-borne disease and sanitation should be observed. The extensive presence of

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microorganisms resistance to antibiotics should also be a concern.

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