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Research Article

Microbial and Molecular Studies of Microbes in Raw Grouper Fish (Hamoor) in Jeddah Province

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Abstract

Jeddah is the second largest city in Saudi Arabia and is located on the Red Sea coast (western seaboard). It has the largest seaport along the Red Sea coast. Rapid industrialization and urbanization in the Jeddah area have raised concerns over potential impacts on public health and the environment. Fish alone accounts for over 80% of the animal protein in our diet. But occasionally, infections or intoxication from eating fish might lead to disease. The general pollution of the aquatic environment is thought to be reflected in it. Fish are conditioned by their surroundings, therefore it stands to reason that if the habitat in which they grow and are harvested is chemically or microbiologically polluted, the fish are likewise polluted. These fish can spread disease to nearby persons through handling while being transported to the landing center and wholesale market. Monitoring the microbial contaminants in commonly consumed fish will thus help safeguard the public from any potential adverse risks. This study isolated several species of bacteria from specific kinds of fish raw Greasy grouper fish (Hamoor collected from the Jeddah central fish market and identified them molecularly. Fish samples were collected individually in a clean polyethylene bag and transferred immediately to the laboratory in refrigerated condition. Then fish samples were dissected, and bacteria were isolated on three media. McConkey agar, TCBS agar, and Blood agar. Extracted DNA of samples isolated 16S rRNA gene sequences of all the (41) bacteria isolated were amplified.

Keywords: Raw Grouper Fish; Media; 16srRNA Gene; PCR; Susceptibility

Introduction

The source of life's energy is food. Humans require food to survive. As a result, there are numerous food sources. Food derived from plants and food received from animals are the two primary categories of food sources. Both of them are important sources of nutrition. One of these two sources provides all of the food that we eat. Meats are animal products that are eaten and used as a direct source of protein. There are two categories: white meat and red meat. Red meat is the highly fatty meat of cows, goats, camels, and sheep. White meat, which is found in chicken and fish, is lower in fat. Compared to red meat, white meat is healthier and easier to digest [1].

Considering this, aquatic food products have been an essential component of the human diet ever since people began fishing in the oceans and raising fish for sustenance in aquaculture. There is such a large demand on the global market for these nutritive goods that more edible aquatic animal food products are trafficked abroad [2]. A statistical report from the Saudi Ministry of Environment, Water and Agriculture revealed that the Saudi average per capita consumption of fish compared to global consumption for 2020-2021, as the rate of consumption per kilogram of fish reached 8.8 kilograms per Saudi individual. In terms of annual consumption and foreign trade of fish in the Kingdom during 2020-2021, the annual fish consumption amounted to 305,145 tons, fish imports amounted to 210,016 tons, and exports amounted to 13,506 tons [3].

A balanced diet must include fish because it has special nutritional and health benefits. More and more focus has been placed on fish as a source of vital nutrients for our diets, particularly as a source of micronutrients and critical omega-3 fatty acids (eicosatetraenoic acid (EPA) + docosahexaenoic acid (DHA), as well as a unique supply of high-quality proteins. Children's brain and nervous system development depend on DHA and iodine, both of which are exclusively present in meals derived from aquatic environments. To maintain the best possible brain development, it is crucial to limit fish consumption among young children, pregnant women, and those who are nursing [4]. But occasionally, the seafood we consume may have an impact on our health. Due to improper handling and storage, exposure to ambient temperatures while fishing, and contamination from handling by hand, the quality of the fish is lost. Food quality and safety, however, are significant global issues today [5].

Foodborne illness causes diarrheal disease, which can have long-term impacts on children's growth and physical and mental development. It also causes many children to die in the developing world and has a significant negative impact on the healthcare systems. Food-borne illnesses are conditions brought on by ingesting bacteria, toxins, or cells made by microorganisms found in food. Depending on the quantity of tainted food consumed and the susceptibility of the person to the toxin, the severity of the signs and symptoms may change. The prevalence of bacteria varies among fish species generally according to biological and environmental conditions. Anthropogenic activities that cause point and non-point pollution in coastal waterways are the main sources of microbial infections in fish. Vibrio is one example of a naturally occurring aquatic disease that can infect humans through food ingestion. Therefore, monitoring bacterial infections may provide early warning to protect seafood consumers from contamination risks [6]. Among the fish eaten commercially. Marine serranid fish (groupers) are abundant in the Red Sea and have commercial importance. It is regarded as one of the most significant fish species in the Jeddah area [7]. Thus, continuous monitoring of microbiological contamination can help to safeguard the general public from any threats.

Materials and Methods

Sampling

Four fish were selected randomly with an average weight of 500 g each from several stores central fish market in Jeddah. Fish samples were collected individually in a clean polyethylene bag kept in the ice box and transferred immediately to the laboratory in refrigerated condition [8].

Sampling processing

Swabbing is a superior method for sampling the microbiota of mucosal surfaces for broad ecological research in fish [9]. Therefore, Samples of gills, skin, and gut of Epinephelus areolatus (Hamour) fish were aseptically obtained by sterile cotton swabs. An opening into the body cavity was carefully made with a sterile scalpel to remove a small portion of the gut for swabbing. and then prepared for plating [10].

Isolation of bacteria

swabs were plated on three different selective media, Macconkey's agar (Mac), Thiosulfate Citrate Bile Salts agar (TCBS), and Blood agar. The plates were numbered and named based on where the fish sample was taken, Skin (SK), Gills (G), Faecal (F). Isolation media were prepared by suspending the Mac (51.5 g/L), and TCBS (89 g/L) in sterile distilled water. Media were completely dissolved by heating on a hot plate and sterilized by autoclaving at 15 lbs pressure and 121 C temperature. for 15 min. TCBS media was not autoclaved but heated until boiling. Media were then cooled up to 50 C and aseptically poured into Petri plates under sterile conditions. isolation of bacteria was done by spread plate zig-zag streak method. The plates were incubated in an inverted position at 37 ± 2 C for 24–40 h [11].

Due to not diluting the isolations in liquid media. originated many colonies mixed in culture. The Individual and different colonies were purified by a sterile inoculation loop and transferred to new media sterile. (Mac) agar, (TCBS) agar, and Blood agar. plates were incubated in an inverted position at 37 ± 2 C for 24–40 h.

Bacterial characterization and identification

Biochemical characterization (Gram staining)

Gram staining is a differential staining method since more than one dye is used as primary and secondary dyes. In gram staining, gram-negative bacteria lose their crystal violet iodine complex and become colorless after washing, but gram-positive bacteria retain the complex and remain purple. The color of the purple gram-positive bacteria isn't affected from safranin, the discolored gram-negative bacteria are dyed pink with safranin. This test differentiates the bacteria into gram-positive and gram-negative bacteria, which helps in the classification and differentiation of microorganisms. In this practice, gram staining procedure are applied on the bacteria after preparation of smear [12].

Microscopic observations of the bacteria isolate were performed using the following method. The bacterial isolate was smeared using an inoculation loop and heat-fixed on the glass slide. Slightly heating the slide has been found to help with cell adhesion and prevent significant loss of bacterial isolate while rinsing. The dried slide was stained with crystal violet for 1 min and washed with indirect tap water. Subsequently, the slide was flooded with iodine for 1 min before being rinsed with tap water. A few drops of 95% ethanol were added to the slide for 5 s and washed with tap water. Following this, the inoculated slide was flooded with safranin and left for 45 s. The slide was washed with tap water and air-dried for a while. The cell shape and type of bacteria were observed using a light microscope with a magnification of 100× [13].

Biochemical characterization (fermentation)

MacConkey agar

It is a selective and differential culture medium for bacteria. It is designed to selectively isolate Gram-negative and enteric (normally found in the intestinal tract) bacteria and differentiate them based on lactose fermentation. Lactose fermenters turn red or pink on MacConkey agar, and no fermenters do not change color. The media inhibits growth of Gram-positive organisms with crystal violet and bile salts, allowing for the selection and isolation of gram-negative bacteria [14].

Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS)

TCBS Agar is used for the selective isolation of Vibrio cholerae and other enteropathogenic vibrios. Thiosulfate and sodium citrate, as well as the alkalinity of the medium, considerably inhibit the growth of Enterobacteria. Ox bile and sodium cholate slow the growth of enterococci and inhibit the development of Gram-positive bacteria. The acidification of the medium resulting from the fermentation of sucrose makes bromthymol blue turn yellow [15].

Biochemical characterization (hemolytic)

Blood agar is an important clinical method. It is both rich and differentiated. Blood contains many nutrients that help support "difficult" bacteria, those that need additional or specific types of nutritional support. Many bacteria can hydrolyze blood, partially or completely, and these patterns of hemolysis can help differentiate and identify some bacterial pathogens. There are two types of hemolysis. Partial hemolysis is termed Alpha hemolysis (α) is caused by damage to (but not breakdown of) red blood cells in the blood Colonies typically are surrounded by a green, opaque zone. Beta-hemolysis(β) is complete hemolysis, is the breakdown of RBCs and the medium appears completely transparent around the colonies. Non-hemolytic bacteria (often called gamma γ-hemolytic) show no lysis or clearance of any kind [16].

Identification of bacteria based on 16s rRNA gene sequencing.

DNA extraction

Inoculate pure bacterial colonies in test tubes contain 5-ml Nutrient broth (NB) sterile, incubated in at 37 ± 2 C for 40 h.

1.5 of culture was to placed into a new 2 mL collection tube. (Eppendorf tube). Spined the tube in a microcentrifuge for 5 min at 8000 rpm or until a pellet forming, Discard the supernatant. This pellet was resuspended 200µl TES buffer (Lysis buffer) and vortixed. Then lysis solution 10µl and proteinase K 10µl were added and mixed homogeneously. (Lysozymes are hydrolytic enzymes characterized by their ability to cleave the glycosidic bonds in peptidoglycan, a major structural component of the bacterial cell wall. This hydrolysis action compromises the integrity of the cell wall. Further, the addition of Proteinase K aids in the deactivation of RNase/DNase and avoids the degradation of nucleic acids in the solution.). Then, complete cell lysis was achieved by incubating

the sample at 56°C for 2h using a water bath. Thereafter incubated at room temperature for 10 min to reduce the reaction. Sodium acetate 250µl were added, to separate nucleic acids from other cellular substances, spined in a microcentrifuge for 5 min at 8000 rpm, appeared pure solution (DNA, RNA, Proteins) was placed into a new 2 mL collection tube.

Depending on amount what was withdrawn of the pure solution. A mixture of phenol, chloroform, and isoamyl alcohol is added to separate nucleic acids from proteins, lipids (17). And put it spin in centrifugation for 5 min at 8000 rpm was done, to get a supernatant, which is the Nucleic acid was placed into a new 2 mL collection tube. after that precipitate the DNA by adding isopropanol to the same amount as the was withdrawn from supernatant. Then Spined the tube in a microcentrifuge for 5 min at 8000 rpm for Discard the supernatant.

Thereafter, 70% ethanol 100µl was added and Spine the tube in a microcentrifuge for 5 min at 8000 rpm for Discard the supernatant. Then TE buffer was added 30µl (The purpose of TE buffer is to solubilize DNA or RNA, while protecting it from degradation).

Then it is stored at -20 for a short time or -80 for a long time.

The agarose gel electrophoresis method

Was performed agarose gel electrophoresis to separate the desoxyribonucleic acid (DNA) fragments following the size. This was achieved by adding 10g of agarose in 1000ml of 1X TBE buffer and microwaved for thirty seconds. Then the mixture was allowed to cold, and ethidium bromide (5uL) was adding and transferred to a tape tray. The combs tips avoided bubbles. The comb was placed and kept for 20 minutes to set. After tapes and comb removal, the sample was mixed 2.5ul of sample with 2.5uL loading dye. The gel was then run at 120V for 30minutes and observed under UV light.

Amplification of the 16S rRNA gene by PCR

First, the concentration of the isolated DNA was measured in the NanoDrop-2000 device (Thermo Fisher Scientific, Waltham, MA, USA). by adding 1µL of the sample. Based on the results that appeared, the measurements were balanced to a concentration of 50ng/ml ± 3. A volume of 10 µL, by a mathematical operation based on a law:

C1 V1 = C2 V2

C1 in Nanodrop V1 = 5010

500/ C1 = V1+ H2O

= 10µL

PCR amplification was conducted in 25µL total volume using 12.5µL of 2X master mix and 0.5µL of each specific forward and reverse primers 1µL of the extracted DNA and 10.5µL H2O.

These amplifications were performed by MultiGene Mini Thermal Cycler device.

The initial step of denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 Second, annealing at 57°C for 30 Second, extension at 72°C for 30 Second, and final extension at 72°C for 10 min. Amplified the 16S rRNA gene using universal primers:

27F (5'AGAGTTTGATCCTGGCTCAG3'),

1492R (5'AAGGAGGTGATCCAGCCGCA3').

Was performed agarose gel electrophoresis to separate the PCR. the PCR was mixed 2.5ul of sample with 2.5uL loading dye. The gel was then run at 120V for 30minutes and observed under UV light. The resulting PCR products were visualized under a UV transilluminator plus was used as the molecular size marker [18].

Analyses of 16S rRNA genes sequencing

For further studies (sequencing) of amplified samples. 16S rRNA genes were sent to Macrogen Korea. Blast search9 was used for the analysis of sequences, and the nearest match was found in GenBank. Finally, the phylogenetic analysis was performed for the blast results.

Susceptibility test of the bacteria to antibiotics

An antibiotic sensitivity test was performed disk diffusion method on nutrient agar (NA), using Mastring-S™ M5 is a ring of antibiotic impregnated discs. Different 6 antibiotic were used such as using the following discs: Ampicillin (AP) 10µg/disc, Augmentin (AUG) 30µg/disc, Gentamycin (GM) 10µg/disc, Cefoxitin (FOX) 30µg/disc, Cephalothin (KF) 30µg/disc, Cotrimoxazole (TS) 25µg/disc.

The ring discs containing (6) antibiotics were placed on each petri dish over the individual isolates and incubated at 37°C. We measured clearance zones (zones of inhibition) using a meter ruler after 48 hours of incubation and classified the isolates as resistant or sensitive.

These types of antibiotics were chosen because they are broad-spectrum. The term broad-spectrum antibiotic can refer to an antibiotic that acts on the two major bacterial groups, gram-positive and gram-negative or any antibiotic that acts against a wide range of disease-causing bacteria.

It means that the antibiotics which kill or inhibit a wide range of harmful or disease-causing bacteria. These are equally effective against gram-positive and gram-negative bacteria (both).

Results

The study's purpose was to evaluate the microbiological situation of raw grouper fish from a central fish market in Jeddah, Saudi Arabia, which could indicate the hygienic state of the fish consumed and significant public health hazards. Additionally, it will be screened for potential pathogenic strains and identified using their 16S rRNA gene sequences.

Isolation of bacteria

An isolated 41 samples were from four (4) raw grouper fish from different shops from the central fish market (S1, S2, S3, and S4), and 15 bacterial strains were isolated from these samples. 4 strains were isolated from (S1), 1strains was isolated from (S2); also 4 strains were isolated from (S3), and 6 strains separated isolated from (S4), However, the largest number of bacteria was found by (S4) with 6 strains, followed by (S3) with 4 strains, (S1) with 4 strains. Furthermore, the lowest bacterial count was recorded by (S2) with 1 strain (Table 1).

Figure 1: The isolation of bacteria.

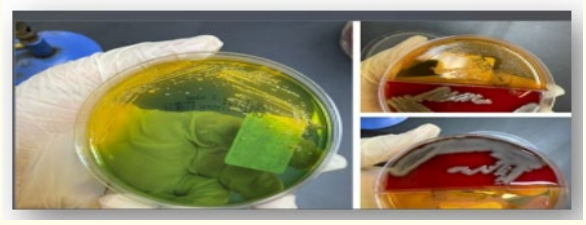

Figure 2: The purification of isolated bacteria.

Biochemical characterization (Gram staining)

In of the 15 isolates that were investigated in this investigation, 8 showed positive Gram stain results. This suggests that the cell walls of these bacteria have a thick covering of peptidoglycan. Conversely, 7 isolates had negative Gram stain results, indicating that the peptidoglycan layer in their cell walls is either absent or very thin. Table 1 provides a summary of these observations.

Source of bacteria	No of isolate	Bacteria isolation	Gram staining
S1	F3	Aeromonas salmonicida	+ve
S1	G6	Bacillus tropicus	+ve
S1	F31	Macrococcus caseolyticus	+ve
S1	Sk33	Mammaliicoccus sciuri	+ve
S2	Sk19	Salinicoccus salsiraiae	+ve
S3	Sk7	Psychrobacter piechaudii	-ve
S3	F8	Providencia rustigianii	-ve
S3	G10	Haemophilus piscium	-ve
S3	Sk17	Bacillus subtilis	+ve
S4	G1	Carnobacterium gallinarum	+ve
S4	Sk9	Aeromonas piscicola	+ve
S4	F11	Psychrobacter sanguinis	-ve
S4	G18	Providencia alcalifaciens	-ve
S4	F22	Pseudomonas deceptionensis	-ve
S4	Sk26	Psychrobacter arenosus	-ve

Table 1: List of bacteria with their isolation source and gram stain.

Molecular identification of the isolated bacterial strains

16S rRNA gene sequences of all (41) bacteria were amplified (Figure) and subjected to 16S rRNA gene sequencing. The results showed that bacterial isolates were mainly members of 10 genus, namely, *Aeromonas, Bacillus, Macrococcus, Mammaliicoccus , Salinicoccus , Psychrobacter , Providencia , Haemophilus , Pseudomonas, and Carnobacterium.* and 15 species, namely, *Aeromonas salmonicida, Bacillus tropicus, Macrococcus caseolyticus, Mammaliicoccus sciuri, Salinicoccus salsiraiae, Psychrobacter piechaudii, Providencia rustigianii, Haemophilus piscium, Pseudomonas deceptionensis, Carnobacterium gallinarum, Bacillus subtilis, Aeromonas piscicola, Psychrobacter sanguinis, Providencia alcalifaciens and Psychrobacter arenosus.* (Table 1). However, the maximum number of bacteria belonged to the genus Psycrobacter (3strains) followed by Aeromonas (2 strains), Bacillus (2 strains), *Macroccus* (2 strains), *Provedincia* (2 strains), Mammalicoccus (1 strain), *salinicoccus* (1 strain), *Haemophiius* (1 strain), Carnobacterum (1 strain) and Psuedomonas (1 strain), respectively. However, the identification of all (41) isolates has been submitted to the GenBank database of NCBI-USA.

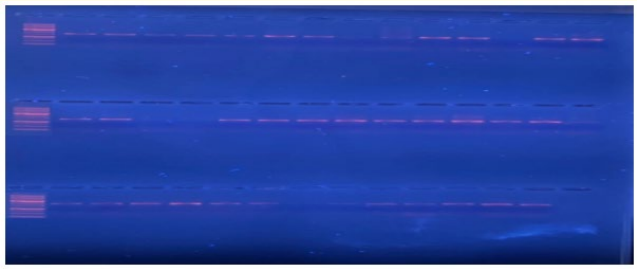

Figure 3: PCR products of 16s rRNA gene of all forty-one 41 isolate samples from raw fishes.

Susceptibility test of the bacteria to antibiotics (Disk Diffusion Method)

Discussion

Fish is generally considered a safe food, and the muscles of healthy fish are considered sterile, although controversy over this continues. It is recognized that microorganisms are commonly

No of bacteria	Susceptibility test of the bacteria to antibiotics					
	KF	TS	AP	AUG	GM	FOX
G18	S	S	R	R	S	S
	22mm	24mm	12mm	13mm	25mm	23mm
F3	R	S	I	S	S	S
	0mm	24mm	25mm	27mm	25mm	33mm
F31	R	S	R	R	S	S
	14mm	21mm	0mm	17mm	17mm	35mm
F8	R	S	R	R	S	S
	0mm	24mm	12mm	0mm	20mm	22mm
SK33	S	S	R	R	S	S
	21mm	26mm	12mm	0mm	28mm	23mm
SK19	R	S	R	R	S	R
	0mm	20mm	0mm	0mm	27mm	0mm
G6	R	S	R	R	S	R
	9mm	0mm	0mm	0mm	22mm	6mm
SK7	I	S	R	R	S	S
	17mm	23mm	14mm	12mm	24mm	25mm

SK17	R	S	R	R	S	S
	0mm	21mm	0mm	0mm	29mm	23mm
G1	S	S	R	I	S	S
	24mm	30mm	12mm	14mm	29mm	25mm
G10	S	S	I	S	S	R
	30mm	35mm	24mm	23mm	27mm	0mm
SK9	S	S	R	S	S	R
	31mm	28mm	20mm	21mm	23mm	9mm
SK26	R	S	R	R	S	R
	0mm	23mm	0mm	0mm	26mm	0mm
F11	R	S	R	R	S	S
	0mm	25mm	0mm	0mm	25mm	31mm
F22	I	S	R	R	S	S
	17mm	24mm	0mm	0mm	24mm	21mm

Table 2: Susceptibility of isolated bacteria to antibiotics.

R = resistance to antibiotic; I = intermediate sensitivity to antibiotics; S = sensitivity to antibiotics.

Abbreviation	Name of Antibiotic	Unit Per Disc	Color
KF	Cephalothin	30	Primrose
TS	Cotrimoxazole	25	White
AP	Ampicillin	10	Grey
AUG	Augmentin	30	White
GM	Gentamicin	10	Salmon
FOX	Cefoxitin	30	White

Table 3: Guidelines chart.

found on the surfaces of fish, such as skin and gills, as well as inside fish in areas such as the digestive tract and internal organs. However, most outbreaks of food poisoning associated with fish are derived from the consumption of raw or undercooked products, Due to unsanitary procedures, alternating duties assigned to workers and handlers, airborne microorganisms during product packing, and poor proper storage. have been implicated in outbreaks of bacterial diseases, and biotoxins, some spoilage bacteria can produce decarboxylase enzymes and convert free histidine into large amounts of histamine, leading to scombroid poisoning, and food safety concerns [19]. On the other hand, with the presence of antibiotic-resistant strains causing human infections, treatment options may become very limited for the patient after his infection.

Molecular phylogeny extends our knowledge regarding organism relationships and provides the foundation for conventional identification techniques. Based upon 16S rRNA gene sequences analysis, strains, DSM-4847, ATCC-33658, MCCC-1A01406, CIP-110584, NCTC-6933, CECT-7443, CIP-106116, DSM-10, DSM-30120, 13983, RH-1, M-1, R-7, ATCC-13548 and DSM-20345 were identified as Carnobacterium gallinarum, Aeromonas salmonicida, Bacillus tropicus, Psychrobacter piechaudi, Providencia rustigianii, Salinicoccus salsiraiae, Haemophilus piscium, Bacillus subtilis, Providencia alcalifaciens, Psychrobacter sanguinis, Salinicoccus salsiraiae, Pseudomonas deceptionensis, Psychrobacter arenosus, Macrococcus caseolyticus and Mammaliicoccus sciuri (Table 1). The 10 bacterial genera isolated in this study are *Bacillus, Aeromonas, Macrococcus , Mammaliicoccus , Salinicoccus , Psychrobacter , Providencia , Pseudomonas, Haemophilus and Carnobacterium*. support and confirm at previous research studies at the local level the presence of three bacterial genera isolated from edible fish in the local fish market in Jeddah [11] (Pseudomonas, Psychrobacteria, and Aeromonas). and four bacterial genera at another previous research in the central fish market in Jeddah [8] confirms the presence of (*Salinicoccus, Psychrobacter, Aeromonas, Pseudomonas*).

Fish samples were collected individually in a clean polyethylene bag and transferred immediately to the laboratory in refrigerated condition. Then fish samples were dissected, and bacteria were isolated on three media. The selective McConkey agar is a differential culture medium for bacteria. It is designed to selectively isolate gram-negative and enteric (normally found in the intestinal tract) bacteria and differentiate them based on lactose fermentation. TCBS agar, PCR results indicated the ability of Aeromonas isolates to grow on TCBS-producing yellow colonies which was complete [20]. Blood agar is an enriched medium used for the cultivation of bacteria that do not grow easily These bacteria are called "fastidious" because they require a special, enriched nutritional environment. Blood agar is used to grow a wide range of pathogens, especially those that are more difficult to grow, such as Haemophilus influenzae, Streptococcus pneumoniae, and Neisseria species. It is also necessary to detect and differentiate hemolytic bacteria, particularly Streptococcus species. It is also a differential medium for detecting hemolysis (destruction of red blood cells) by cytolytic toxins secreted by certain bacteria, such as certain strains of Bacillus, Streptococcus, Enterococcus, Staphylococcus, and Aerococcus subcultured and morphologically identified were examined by Gram stain.

In this investigation, the most prevalent species that were isolated were Aeromonas, Pseudomonas, Psychrobacter, Providencia, and Macroccocus. Some species of the genus Aeromonas are considered to possibly cause gastroenteritis in humans, and these may also be present naturally in the marine or more especially, the estuarine environment (Beyari., *et al.* 2021a). Psychrobacter species are considered rare opportunistic human pathogens in this [21]. As a result, isolated germs are very dangerous to the health of people, animals, and the environment. It highlights the pressing need to enhance the Jeddah fish market's quality assurance and control procedures. Educating the community about dealing.

The antibiotic susceptibility of the isolated bacteria for each specific antibiotic tested. Ampicillin exhibited the highest resistance level, with 86% of the bacterial isolates being resistant, while 14% were sensitive to this antibiotic. Gentamicin and Cotrimoxazole showed the lowest resistance level, with only 0% of the bacterial isolates being resistant, indicating that 100% were sensitive to both antibiotics. Augmentin demonstrated resistance in 73% of the bacterial isolates and sensitivity in 26%. Cephalothin exhibited resistance in 53% of the bacterial isolates and sensitivity in 34%. Cefoxitin and Penicillin-G displayed moderate resistance levels, with 33% of the bacterial isolates being resistant, while 66% were sensitive to both antibiotics.

These results indicate that there is variability in the susceptibility of the bacterial isolates to different antibiotics. The high resistance observed raises concerns about its effectiveness in treating infections caused by these isolates. Conversely, the lower resistance observed for Gentamicin and Cotrimoxazole suggests they may be a more suitable antibiotic option. The presence of resistant bacteria in the isolated raw grouper fish of different is a significant finding as it highlights the potential for the spread of antibiotic-resistant strains within these environments. This emphasizes the importance of implementing proper hygiene practices, prudent antibiotic use, and regular monitoring of antimicrobial resistance patterns to mitigate the risk of infections caused by multi-antibiotic-resistant bacteria.

The results suggest that there is variation in the bacterial isolates' resistance to various antibiotics. Concerns are raised regarding its efficacy in treating infections brought on by these isolates because of the high resistance seen for. On the other hand, the reduced resistance to Cotrimoxazole and Gentamicin indicates that it would be a better antibiotic choice. The identification of resistant bacteria in the raw fish at Jeddah's Central Fish Market is noteworthy because it raises the possibility that antibiotic-resistant strains could spread in these settings. In order to reduce the risk of infections brought on by bacteria that are resistant to several antibiotics, it is crucial to follow good hygiene practices, use antibiotics carefully, and regularly monitor antimicrobial resistance patterns.

Knowing the particular species of bacteria that are present can help determine the possible health hazards linked to particular genera and direct the creation of focused preventative and control measures. It is noteworthy that although 16S rRNA gene sequencing offers significant insights, other techniques for characterization and identification, including whole-genome sequencing or biochemical testing, might be required to ascertain the specific species or strains within each genus.

Overall, the microbial diversity and composition in the Central Fish Market in Jeddah were revealed by the identification and

classification of the bacterial isolates made possible by the combination of Gram staining, PCR amplification of ribosomal DNA, and 16S rRNA gene sequencing. The importance of putting in place appropriate hygiene procedures, using antibiotics sparingly, and routinely observing patterns of antimicrobial resistance.

Conclusion

Bacterial contamination in commercial Raw fish in the Central fish market of Jeddah Province was investigated by culture-dependent method and 16S rRNA gene sequencing of isolates. Results revealed differences in bacterial genera and species from fish parts. 16S rRNA sequences identified some Opportunistic pathogens such as Aeromonas ssp and Psychrobacter ssp were also identified with severe antibiotic resistance for some isolated. The existence of these bacterial species in fish body samples is of concern. The present results indicate the potential risks of bacterial contamination of the fish from the Jeddah area. Periodic monitoring, preferably including both microbial loads and species of concern, is thus important to keep track of any.

The handling of fish by undisinfected hands of consumers creates a high risk of spread of contamination by bacterial diseases in particular those that may reach epidemic status, therefore, Personal hygiene practices are important to save fish from biological hazards that create poisonous foods. very important to keep the fish storage temperature low, preferably around 0°C [22]. Freezing is the cheapest and simplest way to preserve fresh fish. Thus, slowing down the process of microbial and enzymatic corruption. It keeps the fish moist, thus preventing surface drying and weight loss.

Extends shelf life. It must be ensured that quality control is implemented by official bodies and authorities, which in turn will ensure that possible risks in food products are reduced and the safety of consumers is enhanced.

This study shows that molecular techniques such as 16srRNA gene sequencing are a powerful tool for detecting pathogenic bacteria in fish that can be used by regulatory laboratories.

Future research must continue at all stages, to finally ensure high food quality and safety and security for consumers.

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