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Research Article
Evaluation of Pathogenic Microorganisms Isolated from Grey Mullets (Mugilidae) *Valamugil seheli* Muscles Cultivated in Khawr Al-Mukalla Sea Waters, Hadhramaut, Yemen

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Abstract

Background and Objective: Mugilidae aquaculture is found worldwide in coastal temperate and tropical waters. It is globally important industry that provides essential food to a growing world population. This study was carried out to evaluate common types of pathogenic bacteria in the muscles of *Valamugil seheli* of Khawr Al-Mukalla seawater as well as to evaluate the nutritional value of protein, fat ash and carbohydrates of this species. **Materials and Methods:** The trial was undertaken in the 4 seasons of the year from March, 2019 to February, 2020. A total of 144 specimens of fish and 12 triplicate samples of surface seawater were collected and evaluated. **Results:** Significant values ($p<0.05$) were obtained in the total count, *Staphylococcus aureus*, *Escherichia coli* and total coliform. The number of isolated microorganisms in the terms of the bacterial total count, *S. aureus*, *E. coli* and total coliform were 7 log CFU, 3 log CFU, 2.7 log CFU and (500 MPN), respectively. No growth of *Salmonella* sp. and *Vibrio parahaemolyticus* was observed. However, protein, fat, ash and moisture content in the muscles of *V. seheli* throughout the seasons were evaluated. Overall, the highest values of pathogenic bacteria were found in summer while the lowest one in winter. **Conclusion:** The pathogenic microorganisms in the muscles of *V. seheli* and seawater at Khawr Al-Mukalla were in the range scale of Yemen standard.

Key words: Biochemical composition, Khawr Al-Mukalla, mugilidae, microorganisms, Yemen standard

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Grey mullets (Mugilidae) fish are economically significant in most areas of the ecosphere. They are also cultured in many regions of the world in both extensive and intensive systems[1]. Egypt is considered the greatest producer of cultured Mugilidae, producing 84% of the total world of the Mugilidae by aquaculture production[2]. The fish contains high nutritional components such as protein, lipid, vitamins and minerals as well as good digestibility, high palatability and energy source[3].

Bacterial infection is one of the major problems in aquaculture which leads to heavy losses and causes a great drop in fish production in industry[4]. Most of the bacteria associated with these diseases are naturally saprophytic organisms and are widely distributed in the aquatic environment[5].

Pathogenic bacteria which spoiled fish are *Vibrio* sp., *Shigella* sp., *Salmonella* sp., *Streptococci* sp., *Staphylococci* sp., *Listeria* sp., *Clostridium* sp and coliforms[6]. Some reports suggested that the consumption of microbiologically spoiled kinds of seafood might be responsible for food-borne diseases like diarrhoea, salmonellosis, shigellosis, cholera and even some neurological diseases[7-9]. On the other hand, other researchers reported that Mugilidae fish are affected by pathogenic microorganisms during their transportation and storage[5,10]. *Valamugil seheli* species are consumed by the people of Al-Mukalla city (Hadhramout-Yemen) as the main source of seafood in the meals. Consequently, For human utilization, this species should be free from pathogenic microorganisms or in the acceptable range of Yemeni standard[11,12].

The present study aimed to detect and identify the common types of pathogenic microorganisms in *Valamugil seheli* muscles and also determine the body composition (fat, protein, moisture ash and carbohydrates) of the same species. In addition, the seawater in the same area of fish's cathead in Khawr Al-Mukalla Hadhramaut, Yemen was evaluated.

MATERIALS AND METHODS

Study area: This study was carried out in Khawr Al-Mukalla, Mukalla, Hadhramaut Governorate-Yemen. This area is situated on longitude "49°07'36.2"E and latitude "14°32'08.0" N which is 1.88 km length and 0.06 km width. The fish and seawater samples were collected from the middle of Khawr Al-Mukalla connected to open seawater.

Fish samples: Through the study, 144 specimens were collected from March, 2019 to February, 2020. About 12 specimens were collected in the 1st week of every month for 12 months using the traditional method. The fish length was in the range of 11-25 cm and the weight was between 43.6-218.8 g. The collected fish were transported in a cool icebox to the Microbiology Laboratory/Faculty of Environmental Sciences and Marine Biology, Hadhramaut University-Yemen, for microbiology and body composition analysis.

Water samples: Seawater samples were collected from the same area of Khawr Al-Mukalla station and during the time of fish's collections. The samples were collected from a depth of about 50 cm beneath the surface in 100 mL sterile bottles. The samples were transported immediately to the laboratory in a cool icebox.

Microbiological test: The fish flesh was used for microbiological tests after removing the skin and homogenous using peptone water. The fish flesh was evaluated by total count, total coliform, *Vibrio parahaemolyticus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. Ten grams of fleshy fish were mixed with 90mLof peptone water. Tenfold serial dilution (10^{-1}-10^{-5}) was conducted of the homogenate mixture using buffered peptone water.

For total coliform and *E. coli*, 100 µL of each dilution was mixed with 10 mL of MacConkey broth (Himedia-LQ115- India) and incubated at 37°C for 24 hrs. Positive results (lactose fermentation and presence of gas) were recorded and the Most Probable Number (MPN) was calculated. On the other hand, for total bacterial count and *S. aureus* isolation, pour plate technique was performed by adding 1 mL of each dilution in a petri dish, then 20 mL of plate count agar (Himedia-SP091G-India) and Baird-Parker agar (Himedia- M043 -India) was poured for total bacterial count and *S. aureus* isolation, respectively. The plates were incubated at 37°C for 48 hrs. Similarly, for *Vibrio parahaemolyticus* and *Salmonella* sp. isolation, the spreading technique was conducted. 100 µL of the homogenous mixture was added on each plate of thiosulfate citrate bile salts sucrose agar (TCBS) (Himedia - M870S– India) and xylose-lysine deoxycholate agar (XLD) (Himedia - MU031– India). The plates were incubated at

37°C for 48 hrs. The colonies were counted gram stain technique and catalase test were conducted for all cultured plates[13-15].

Water evaluation: One mL from the water sample was added to the tubes containing 9 mL of lactose broth medium (Himedia-M1003-India) containing inverted Durham tubes for collected gas bubbles. *Escherichia coli* and coliform bacteria were isolated and the number was detected using the Most Probable Number (MPN) method[16].

Body composition: The body composition of fish containing moisture content, protein, fat and ash were analyzed using the association of official analytic chemist's methods[17,18].

Statistical analysis: The data were analyzed using one-way ANOVA software. The mean differences between treatments were tested for significant differences ($p<0.05$) using Duncan's multiple range test. All statistical analyzes were performed using the SPSS software package, version 21.

RESULTS

Khawr Al-Mukalla is one of the famous places in Al-Mukalla city, Hadhramaut Governorate, Yemen. It is widely used for fishing. Sofar, there is no comprehensive research conducted to evaluate the presence of pathogenic bacteria in the species *Valamugil seheli* as well as the water of Khawr Al-Mukalla.

Evaluation of pathogenic microorganisms in *Valamugil seheli*: The results of bacterial types in *V. seheli* showed that there were significant differences ($p<0.05$) in the bacterial total count, total coliform, *Escherichia coli* and *Staphylococcus aureus* except for *Vibrio parahaemolyticus* and *Salmonella* sp. These result showed that the highest value of total bacterial count was in summer (4.3 log CFU g^{-1}), whereas the lowest value was achieved in winter (3.18 log CFU g^{-1}). The result of total coliform showed that the highest value was found in summer (188 MPN), whereas the lowest value was achieved in winter (21.2 MPN). The highest values of *S. aureus* and *E. coli* were in summer, while the lowest values of *S. aureus* and *E. coli* were in autumn and winter, respectively. However, *Vibrio parahaemolyticus* and *Salmonella* were not isolated throughout all the seasons of the year as shown in Table 1.

Evaluation of pathogenic microorganisms in Khawr Al-Mukalla water: The results of bacterial types from Khawr Al-Mukalla water were analyzed to evaluate microbial pollution during the seasons: Winter, autumn, summer and spring. The result showed that there were significant differences ($p<0.05$) in the bacterial total count, total coliform, *Escherichia coli* and *Staphylococcus aureus* except for *Vibrio parahaemolyticus* and *Salmonella* sp., during the seasons (winter, autumn, summer and spring) from Khawr Al-Mukalla seawater as shown in Table 2. The highest value of bacterial total count was in spring (6.90 log CFU mL^{-1}), whereas the lowest value was achieved in winter (5.36 log CFU mL^{-1}). Similarly, the result of total coliform showed that the

Table 1: Evaluation of bacterial total count, total coliform, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Salmonella* sp. in *Valamugil seheli* muscles during the year seasons

Seasons	Total count (log CFU g^{-1})	Total coliform (MPN)	*E. coli* (MPN)	*S. aureus* (log CFU g^{-1})	*V. parahaemolyticus* (log CFU g^{-1})	Salmonella (log CFU g^{-1})
Spring	4.10 ± 0.5^{ab}	110 ± 1.5^{b}	17.9 ± 3.0^{b}	2.5 ± 0.4^{ab}	Nil	Nil
Summer	4.28 ± 0.9^{a}	188 ± 3.2^{a}	23.8 ± 2.5^{a}	3.3 ± 0.6^{a}	Nil	Nil
Autumn	3.63 ± 0.4^{c}	34.2 ± 2.7^{ac}	5.3 ± 0.8^{c}	2.5 ± 0.6^{ab}	Nil	Nil
Winter	$3.18\pm0.6^{c,d}$	21.2 ± 2.5^{d}	4.5 ± 0.5^{d}	1.0 ± 0.01^{c}	Nil	Nil

Results are expressed as Mean±SE of 3 replicate determinations each season, mean values with different superscript letters in the same column indicate a significant ($p<0.05$) difference

Table 2: Evaluation of bacterial total count, total coliform, *Escherichia coli*, *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Salmonella* sp., in Khawr Al-Mukalla water during the year seasons

Seasons	Total count (log CFU g^{-1})	Total coliform (MPN)	*E. coli* (MPN)	*S. aureus* (log CFU g^{-1})	*V. parahaemolyticus* (log CFU g^{-1})	Salmonella (log CFU g^{-1})
Spring	6.90 ± 0.80^{a}	23.0 ± 0.1^{a}	6.3 ± 1.3^{a}	2.64 ± 0.6^{ab}	Nil	Nil
Summer	6.78 ± 0.91^{ab}	13.6 ± 2.3^{b}	3.0 ± 1.0^{b}	2.93 ± 0.4^{a}	Nil	Nil
Autumn	5.53 ± 1.01^{c}	6.3 ± 1.3^{c}	0.6 ± 0.6^{c}	1.5 ± 0.6^{c}	Nil	Nil
Winter	5.36 ± 1.1^{cd}	4.0 ± 1.0^{d}	0.6 ± 0.6^{c}	1.0 ± 0.01^{c}	Nil	Nil

Results are expressed as Mean±SE of 3 replicate determinations each season, mean values with different superscript letters in the same column indicate a significant ($p<0.05$) difference

Table 3: Body composition of *Valamugil seheli* during the four seasons

Seasons	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)
Spring	78.29±0.03[a]	15.55±0.42[c]	4.35±0.55[c]	1.69±005[c]	0.97±0.03[a]
Summer	78.09±0.19[a]	15.40±0.23[c]	3.90±0.24[d]	1.68±004[c]	0.92±0.18[b]
Autumn	76.38±0.17[b]	16.14±0.11[b]	5.00±0.13[b]	1.71±0.002[b]	0.77±0.14[c]
Winter	74.93±0.13[c]	17.13±0.23[a]	5.32±0.10[a]	1.74±0.003[a]	0.94±0.28[b]

Results are expressed as Mean±SE of 3 replicate determinations each season, mean values with different superscript letters in the same column indicate a significant ($p<0.05$) difference

highest value was found in spring and the lowest value was achieved in winter with MPN values 23.0 and 4.0, respectively.

The highest value of *E. coli* was in spring and the lowest value was winter and autumn, the MPN was 6.3, 0.6 and 0.6 for spring, winter and autumn, respectively. However, *Vibrio parahaemolyticus* and *Salmonella* were not isolated throughout all the seasons of the year. The presence of *E. coli* in Khawr Al-Mukalla seawater was identified due to occurrence of the turbidity, positive indole and production of acid/gas.

Body composition of *Valamugil seheli*: The body composition of *Valamugil seheli* including moisture, protein, lipid ash and carbohydrate during the seasons of a year were evaluated. The results showed that there were significant differences ($p<0.05$) in the body composition of *Valamugilseheli* as shown in Table 3. It was clear that the highest value of protein, lipid and ash were achieved in winter with 17.13, 5.32 and 1.74%, respectively whereas, the lowest value was in summer with 15.4, 3.9 and 1.68% for protein, lipid and ash, respectively. On the other hand, the highest value of moisture and carbohydrate were achieved in spring with 78.3 and 0.97% for moisture and carbohydrate, respectively. However, the lowest value of moisture and were in winter with 74.93%, on the other hand, there was no significant value ($p>0.05$) of carbohydrate in spring, summer and winter with a slight Observation on the lowest value of carbohydrate in autumn with significant difference ($P<0.05$) with other seasons.

DISCUSSION

In the present study, total bacterial count, total coliform, *E. coli* and *S. aureus* showed the same pattern in *Valamugil seheli* fish. The highest values of these microorganisms were in summer, while the lowest were in winter. This result indicated that the contamination of Khawr Al-Mukalla was higher in the summer session. The source of this contamination was non-treated sewage. The contaminated sewage was draining off into Khawr Al-Mukalla in summer in high quantity compared to winter due to using a lot of water during these hot months by residents of Al-Mukalla. In addition, tropical and sub-tropical fish grown in the temperature 30-35°C, this range of temperature was also the optimum temperature for pathogenic microorganisms.

Bacterial total count was performed to give an idea about contamination and it is supported by others[19-21]. Total bacterial count was increased in summer and spring while decreasing in winter[22-24].

Overall, the results of the total count in the present study (4.28 log CFU g^{-1}) was in the agreement with other researchers who mentioned the acceptable total aerobic bacteria count in the fish sample was 6 .0 log CFU g^{-1} or less[25,26]. However, it is reported that the acceptable range of the total count is fish samples 7.0 log CFU g^{-1} or below[27,28].

The presence of total coliform was the indicator of human waste contamination. It was reported by other researchers that the level of contamination by coliform bacteria in fish depends on the extent of pollution in the water[29-34].

The result of this study has showed that coliform bacteria increased in spring and summer while decreased in autumn and winter. This result correlated with the previous result reported by many other researchers[35-38]. *Escherichia coli* is increased when the temperature of the water is increased and decreased when the temperature is decreased. The researchers reported that *E. coli* increased when fish inhabit polluted waters and it was likely that fish could pick up *E. coli* strains and carry them as transient or resident flora to other sources of water[39-41]. The presence of *E. coli* in the fish muscles has made evident the high pollution produced by the sewage discharges[42,43]. The presence of *E. coli* in fish muscle was a good indicator of human beings' contaminations and this was supported by others[44-46]. The number of *S. aureus* in 1 gram of *Valamugil seheli* muscles during the year seasons was in the range 1.0-3.3 log CFU g^{-1}. This finding was comparable to other reports[47-49]. In the current study, the result showed that *Vibrio* sp. and *Salmonella* sp., in *V. seheli* not detected. This result was consistent with other researchers[47,49-51]. On the other hand, *Salmonella* and *V. parahaemolyticus* were not detected in the *V. seheli* samples cultivated in different countries[52-55].

The bacterial total count, total coliform and *E. coli* from Khawr Al-Mukalla water had the same pattern as in *V. seheli*. Total bacterial count in Khawr Al-Mukalla water during the year seasons was in the range 6.9-5.3 log CFU g^{-1}, on the other hand, the number of *S. aureus* bacteria in Khawr Al-Mukalla water during the year seasons was in the range 2.9-1.0 log CFU g^{-1}. These results were comparable to other reports[47,49].

The biochemical body composition (moisture, protein, lipid ash and carbohydrate) of *V. seheli* may have differed regarding the fish age, feeding, reproductive cycle and spawning. Also, there was a relationship between the protein, fat and moisture content versus the age of fish. The value of protein and fat content increased while the moisture content decreased as supported by Kim *et al*.[56], who reported that the percentage of fat and protein content increased while the water content reduced as the body weight increased, in addition, protein ash and fat increase when moisture decreases[57,58]. The highest value was obtained in winter while the lowest value gotten in summer. These observations probably indicated that the biochemical body composition served as a general indication of the nutritional status as supported by Heinsbroek *et al*.[59] and Yousif *et al*.[60], who reported that any seasonal change may lead to alteration in the body composition during growth. During the period of feeding, the protein of muscle tissue increased slightly, fish may have starvation periods for natural or physiological reasons (spawning or migration) or because of external factors such as shortage of food as mentioned by Ashwini *et al*.[61]. During the post-spawning season, increasing the protein content indicating that there was a voracious feeding by the fishes after spawning[62].

Researchers reported that proteins and fat accumulate in gonads when fish matures and during the spawning, the gonadal elements get released either as eggs or milt carrying the protein and fat along with them and protein and fat decline[63,64]. During the post-spawning season, a rise in the protein content was observed which indicates that the voracious feeding by the fishes occurred after spawning[65,66]. These results were in agreement with the present study.

CONCLUSION

Total plate count, coliform *E. coli* and *S. aureus* were isolated from *Valamugil seheli* muscle cultivated in Khawr Al-Mukalla seawater, while *V. parahaemolyticus* and *Salmonella* sp. was not detected. The number of isolated bacteria from the muscles of *V. seheli*, were in the range scale of Yemen standard 2002. It is recommended that the treatment of sewage before their draining into Khawr Al-Mukalla is necessary to save the aquatic ecosystem and to reduce the pollution of water. Further studies are necessary to monitor the faecal pollution in this area.

SIGNIFICANCE STATEMENT

This study discovers the number of pathogenic bacteria present in *Valamugil seheli* muscles cultivated in Khawr Al-Mukalla have Hadhramaut, Republic of Yemen and evaluated these numbers with Yemen standard scale range. In addition, this is the First study describing the nutritional compassion of *V. seheli* in Khawr Al-Mukalla, Yemen.

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