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Article

Hunting of Some Bacterial Species Based on Isolation and Identification from River Water and Sediments and from Tilapia (*Oreochromis Niloticus*) in Al-Qadisiyah Province, Iraq

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Abstract: Bacterial presence or contamination in food is a major alarm for public health. Controlling measures should be taken to limit this problem for better food safety. This procedure starts with some critical steps, such as identification of bacterial species and their pathogenicity. According to these, this investigative study was carried out to explore the existence of some bacterial species in Tilapia fish (*Oreochromis niloticus*) and river water and sediment in Al-Qadisiyah Province, Iraq. For that, 80 live-fish samples were purchased from different regions in the province, which included Al-Saniyah, Al-Dagharah, Al-Shafeiyah, and Al-Diwaniyah City (markets). Water was subjected to physiochemical tests that included pH, dissolved oxygen (% and g/l), salinity (ppt), conductivity (ms/cm), tds (g/l), and temperature. The fish samples were subjected to visual tests, including firmness and clarity of the eyes and color of the gills. Water and fish skin, gills, and gut were used to explore bacterial presence by using conventional cultivation and biochemical tests. The pathogenicity was estimated by the capability of the isolated bacteria to synthetize biofilm. The findings of the physiochemical tests showed no major differences among regions. The findings of the bacterial isolation and identification from water, sediments, and fish samples revealed the presence common bacterial microorganisms shared among regions and the source of samples, which included *Vibrio* spp, *Aeromonas* spp, *E. coli*, *Enterobacter* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Klebsiella* spp, *Salmonella* spp, and *Serratia* spp. The results of the biofilm capability demonstrated that all bacterial isolates were able to produce biofilm; however, *Vibrio* spp, *Aeromonas* spp, *Pseudomonas* spp, and *Serratia* spp showed the highest (p<0.05) in generating biofilms. The data of the current work indicates high levels of bacterial occurrence in fish and their environment with similar bacterial microorganisms.

Keywords: food-borne diseases, food poisoning, food safety

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1. Introduction

Food safety can be understood as a major part of public health and it aims at preventing the diseases derived from unsafe food which may occur at any point in time within the food chain, from production to consumption. Foodborne disease (FBD) is an important public health problem of both developed and developing nations [1]. Almost 600 million individuals get sick every year as a result of consuming contaminated food, about 420,000 of whom die due to FBD every year. Firstly, the World Health Organization (WHO) has reported that about 10 per cent of the global population suffers from FBDs. Secondly, according to a Global Burden of Disease Study done by the WHO in 2010, foodborne infectious diseases affected about 550 million individuals and about 230,000 people died due to this, globally. However, it is difficult to determine the exact mortality caused by FBDs.

Quantifying the burden of FBDs is difficult because the toxic elements that cause them are not necessarily transmitted through food [2], [3], [4], [5], [6].

Scary yet true, consumption of contaminated food is directly responsible for approximately 70 per cent of the cases of diarrheal diseases in developing countries. Low- and middle-income countries suffer high rates of foodborne illnesses due to poor hygienic practices, absence of clean drinking water, contamination and improper storing of food and absence of food safety education [7]. The incidence rate of FBDs in low-income countries is particularly severe owing to the interplay of pervasive issues such as poor sanitation, ineffective food safety enforcement regulations, weak regulatory structures, improper handling of raw food, unsafe and improper temperature control, lack of storage facilities, inadequate cooking practices, poor personal hygiene standards, use of techniques such as cross-contamination of cooked food with raw food and poor food handling [8], [9].

It seems institutions that have a high-density population may experience potential FBDs since outbreak in such establishments could affect a large number of people at the same time. Food handlers should always follow good hygiene practices to reduce chances of cross-contamination and foodborne illness in consumers. Sickness is often caused by poor personal hygiene so food handlers should have information and be practiced in food handling. Hygiene is important in most developing countries because the burden of diseases that are preventable by good hygiene practices are very high [10], [11], [12], [13].

Through poor personal hygiene and poor awareness of key prevention practices, food handlers can contaminate food they work on with various intestinal bacteria, viruses, helminths, and protozoa. Food handlers present a risk of contamination in restaurants: this can occur through poor hygiene in bathroom or when handling food, as well as inadequate awareness of key prevention measures. Such handlers may pass on various pathogens through food they handle [14], [15].

Fish and its products are very important and nutritious foods consumed worldwide. They contribute about 15-20% of total animal protein consumption globally. Many studies have shown the beneficial effects to health in eating fish. This is caused by the presence of proteins, minerals and vitamins; but also, by peptides, amino acids, selenium, and long-chain polyunsaturated fatty acids. Besides nutrition, eating fish food has been associated directly with cardiovascular disease (CVD) prevention, promoting fetal and child development and with beneficial effects in prevention of different other human illnesses and clinical conditions as well [16], [17], [18]. The main health drawback of eating fish is the risk of bacterial contaminations, but also other biological, chemical, and physical contaminations are associated with consumption of fish food. The most significant risk associated with fish foods is of microbiological nature. Fish food may be the cause of food poisoning – bringing with it a wide variety of health issues. This varies from weak discomfort to long-lasting or even fatal sickness. Microbial contamination not only entails health implications for the eaters of food, but also in the process of spoilage of food as well [19], [20], [21], [22].

Bacterial presence or contamination in food is a major alarm for public health. Controlling measures should be taken to limit this problem for better food safety. This procedure starts with some critical steps, such as identification of bacterial species and their pathogenicity. According to these, this investigative study was carried out to explore the existence of some bacterial species in Tilapia fish (*Oreochromis niloticus*) in Al-Qadisiyah Province, Iraq.

2. Materials and Methods

2.1. Samples

This investigative study was carried out to explore the existence of some bacterial species in Tilapia fish (*Oreochromis niloticus*) and river water and sediment in Al-Qadisiyah Province, Iraq. For that, 80 live-fish samples were purchased during early morning hours from different regions in the province, which included Al-Saniyah, Al-Dagharah, Al-Shafeiyah, and Al-Diwaniyah City (markets). These samples were placed in thermal containers and immediately transported to the College of Veterinary Laboratory, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.

2.2. Physiochemical water and fish properties

Water was subjected to physiochemical tests by utilizing a hand-held multi-probe ysi model 600qs (ysi, United Kingdom), which included pH, dissolved oxygen (% and g/l), salinity (ppt), conductivity (ms/cm), tds (g/l), and temperature. The method was followed as mentioned by Sangiorgio et al. [23]. The fish samples were subjected to visual tests, including firmness and clarity of the eyes and color of the gills as described by Chamberlain and G. Titili [24].

2.3. Bacterial isolation and identification

Water and fish skin, gills, and gut were used to explore bacterial presence by using conventional cultivation and biochemical tests. The methods were applied as read in [25]. These organs were swabbed separately for cultivation in duplicates.

Different media types were used, such as nutrient agar, MacConkey agar, brilliant green, and SMAC agar. Bacterial cultures were incubated overnight for later purification. The identification was conducted by colony count, gram staining, and biochemical (catalase, oxidase, coagulase, indole, citrate, and mannitol) tests based on Bergey's Manual of Systematic Bacteriology [26] and Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2016).

2.4. Biofilm activity

TSA-based 10ml cultivates were assessed for the ability to produce biofil by employing a 96-well microtiter plate and according to Djordjevic et al. [27] using a few modifications. These bacterial isolates were 37 °C-overnight-incubated. Later, a 0.5 McFarland standard (1.5 x 107 cells/mL) of dilutions were made. Later, in each well, 200 µL aliquots were employed. Then, aerobic (24hrs-37 °C) incubation was performed. After that, 200 µL of 0.9 % saline was used for washing steps. The mix was then stained with 200 µL 1% crystal violet solution for 20 mins. Later, 200 µL of 95% ethanol was used to release any remaining bound dye. Finally, each well was measured for its optical density (OD) at 570 nm. Each bacterial isolate was subjected to a triplicate setting. The isolates were assessed according to the OD intensity to strong producers (ODi ≥0.12), moderate producers (ODi = 0.1≤0.12), weak producers (ODc ≤ ODi ≤0.1), non-producers (ODi ≤ ODc)(28-30).

3. Results

The findings of the physiochemical tests showed no major differences among regions (Table 1).

Table 1. Physiochemical properties of water where fish was collected

Physical and chemical parameters	Locations	Mean	SD	T-value (0.05)
Temperature	Al-Saniyah	30.31	1.085	0.020
	Al-Dagharah	28.47	0.751	
	Al-Shafeiyah	23.97	2.923	
	Al-Diwaniyah	23.29	2.851	
Conductivity	Al-Saniyah	32.12	0.104	0.001

	Al-Dagharah	41.48	0.880	
	Al-Shafeiyah	32.48	4.013	
	Al-Diwaniyah	41.35	1.204	
Salinity	Al-Saniyah	26.37	1.951	0.472
	Al-Dagharah	21.27	0.142	
	Al-Shafeiyah	23.18	2.952	
	Al-Diwaniyah	22.25	1.032	
Dissolved oxygen	Al-Saniyah	3.640	0.106	0.728
	Al-Dagharah	4.423	0.372	
	Al-Shafeiyah	3.593	0.456	
	Al-Diwaniyah	4.410	0.455	
PH	Al-Saniyah	7.850	0.612	0.238
	Al-Dagharah	7.380	0.177	
	Al-Shafeiyah	7.833	0.462	
	Al-Diwaniyah	7.537	1.099	

The findings of the bacterial isolation and identification from water, sediments, and fish samples revealed the presence common bacterial microorganisms shared among regions and the source of samples, which included *Vibrio* spp, *Aeromonas* spp, *E. coli*, *Enterobacter* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Klebsiella* spp, *Salmonella* spp, and *Serratia* spp (Figure 1).

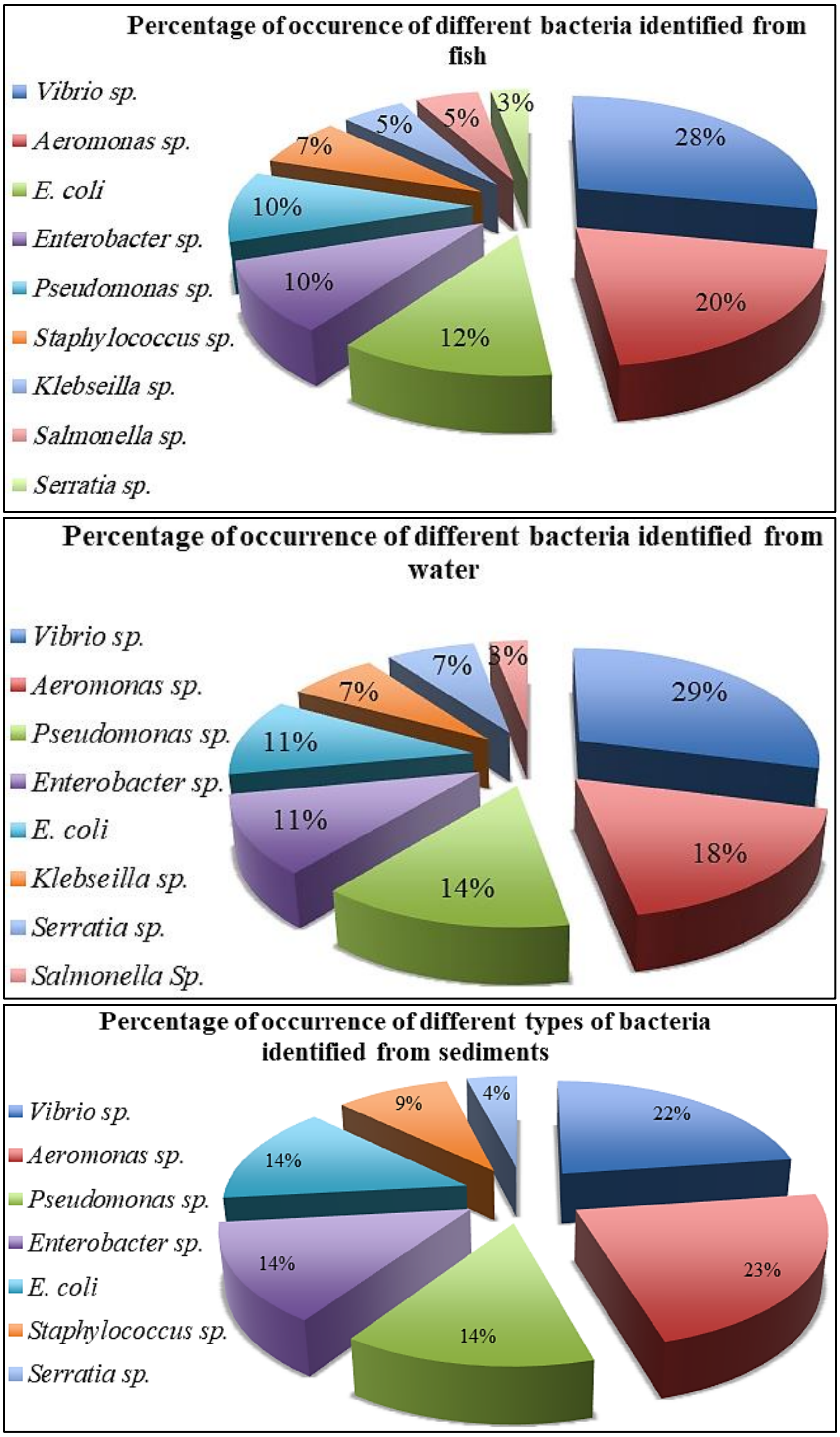

Figure 1. Bacterial isolates from fish, water, and sediment

The results of the biofilm capability demonstrated that all bacterial isolates were able to produce biofilm; however, *Vibrio* spp, *Aeromonas* spp, *Pseudomonas* spp, and *Serratia* spp showed the highest (p<0.05) in generating biofilms (Figure 2).

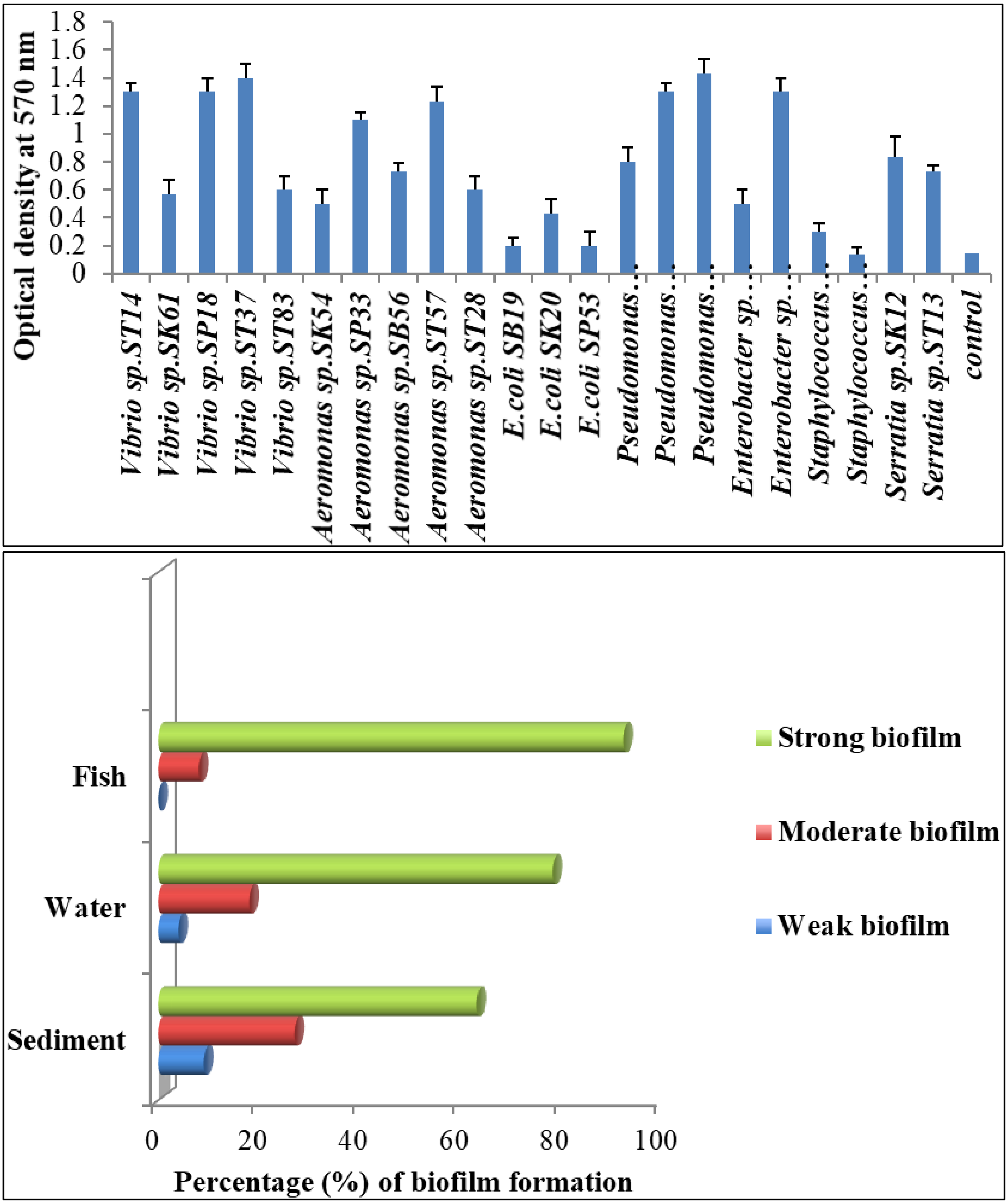

Figure 2. Biofilm formation activity by bacterial isolates from fish, water, and sediment

4. Discussion

Given the increasing popularity of using fish-based food as an excellent source of proteins for preparation of healthy meals with its apparent health benefits, it has become imperative to assess the significant scientific evidences on microbial risks to public health. These analyses have shown a high degree of bacterial contamination fish samples.

The result from this study revealed higher of *E. coli* prevalence than those by Awot et al. [28] study. Nine (9.4%) from fish meat retailing shops in Ethiopia. This study agrees with the findings of Gupta et al. [29], in which 12.96% were contaminated with *E. coli*. However, the result of this study is lower than the result of Wendwesen et al. [30] in which

42.5% of raw fish samples were contaminated and collected from markets in Ethiopia. The result of this study is higher than those by Wendwesen et al. [30], who found that 7.5% of Nile tilapia fish were contaminated. Hanson et al. [31] stated that prevalence of *E. coli* infection was more in plankton feeders (Nile tilapia species) than in catfish.

The result of *Salmonella* prevalence in this study is less considering than research in which (12%) was found in China [32], [33]. Accordingly, the differences in the prevalence may be associated with the nature of water, its quality and environmental characteristics (rainfall, higher temperature, sewage effluents, agricultural run-off, and direct fecal contamination [34].

The occurrence of *S. aureus* found on this occasion was around 9%. This outcome generated a lower finding in comparison with other researchers in different parts of the world. In Egypt, Mohammad et al. [35] reported 31.8% and Murugadas et al. [36] reported 36.5%. Oh et al. [37] reported that the percentage of *S. aureus* was 17.7% in Korea, Haiffa [38] reported that the percentage of S. aureus was 19.8% in Mosul City (Iraq).

Aeromonas spp is an important pathogen in fishes. *A. hydrophila* is a fish and human pathogen and can cause human gastroenteritis, septicemia, and wound infections following trauma or exposure to water. The wide distribution of this pathogen in the aquatic environment, together with its ability to survive and thrive at food chain components, including cold temperature renders this organism as an important food safety pathogen. *A. hydrophila* is commonly isolated from fish and fish products. Prevalence ranged from 0.8% in retail frozen tilapia from Mexico City [39] to 47% in farmed fishes from Egypt [40].

One study found that A. hydrophila was present in 10 to 16% of tilapia [41]. The location in fish where A. hydrophila can be found also vary depending on the fish species. A study in Egypt demonstrated that *A. hydrophila* was present in 6.3% of the viscera samples collected from the retail markets, but *A. hydrophila* was not detected in the muscle samples [42]. Some of these fish carried pathogenic species of *Vibrio alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, and *V. cholerae*. A total of 179 Gram-negative bacteria isolated from waste water showed the presence of *Vibrio* spp and *Klebsiella* spp at (3.91%) and (1.67%), respectively [43].

5. Conclusion

The data of the current work indicates high levels of bacterial occurrence in fish and their environment with similar bacterial microorganisms. The study indicates that most bacterial species were common among regions and the source of samples, such as *Vibrio* spp, *Aeromonas* spp, and *E. coli*, etc., with their ability to produce biofilm in a high percentage, especially *Vibrio* spp, *Aeromonas* spp, and *Pseudomonas* spp. This probably ensures that these bacterial species could be highly pathogenic to animals and humans.

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