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Characterization of culturable bacterial community associated with Thunnus tonggol (longtail tuna) and Caranx sem (blacktip trevally) marketed in the fish market of Muscat (Oman)

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***Abstract*---**The present study was conducted to evaluate the freshness of two Omani fishes; *Thunnus tonggol* (Longtail Tuna) and *Caranx sem* (Blacktip trevally), marketed in the fish market of Muscat, through the characterization of the bacterial community as an indicator for fish

quality. Therefore, 15 fishes from each type were collected from Muscat fish market during the period between September 2019 and October 2021. Skin surface of the fish was examined. Ten grams of each fish were cut from skin surface under aseptic conditions. The cut samples were homogenized and diluted in sterile water. Samples were then cultivated and identified by routine biochemical identification tests and confirmed by the identification system EPI20E when appropriate. A total of 13 bacterial species were detected including *A. hydrophila, A. salmonicida, C. freundii, E. piscicida, E. tarda, H. alvei, L. garvieae, S. marcescens, Y. ruckeri, E. coli, K. pneumonia, P. aeruginosa* and *S. aureus*. Among the thirteen bacterial specie, *A. hydrophila, A. salmonicida, Y. ruckeri, E. coli, K. pneumonia, P. aeruginosa* and *S. aureus* were found to be common in all collected samples. Some of the detected species (like *E. coli*) may indicate that the raw fish sold in Muscat fish market has been exposed to contamination during marketing, which strongly suggests more efforts to improve the quality control systems in Muscat fish market.

***Keywords*---**bacterial community, Thunnus tonggol, Caranx sem, Oman**.**

Introduction

The Sultanate of Oman is located in the southeast corner of the Arabian Peninsula with a total surface area of approximately 309,500 km^2 and comprises continental land and the main islands of Masirah and Al-Halaniyat. Oman has more than 3,165 km of coastline stretching from Musandam in the north to Salalah in the south. Oman overlooks the Indian Ocean, Sea of Oman, Persian Gulf and Arabian Sea. Oman has nine administrative regions of which six are coastally located. The whole coastal belt represents a rich and valuable source of renewable natural resources of which fisheries from Omani waters provide the second major source of income and livelihood option for the Omani population after oil revenues. The Omani ocean shelf along the coastline has excellent natural conditions featuring an unpolluted coastline. Consequently, the marine environment was sustainable for fish diversity, including a variety of staple and financially rewarding species as well as rare and valuable delicacies, such as kingfish, lobster and abalone.

Fish is the one of the most important sources of protein providing about 16% of animal protein consumed by the world populace. It is estimated that about one billion people worldwide rely on fish as their primary source of animal protein (Muhammad et al. 2020). Fishes are widely preferred food all over the globe and cheap source of protein in many developing nations (Tacon and Metian, 2013). At present there are over 20,000 known species of edible fish, shellfish and sea mammals, however, sea food has long been association with transmission of diseases (Ibrahim et al. 2014). Microbiological quality of fish and shellfish has been a matter of great public health concern at both for domestic and international markets. The contamination harms include the presence of

pathogenic microorganisms which causes health problems to the consumers (Mhango et al. 2010).

Equally, fishes provide a suitable natural habitat for certain bacteria such as representatives of the marine genus *Vibrio* that is indigenous to marine environment and sometimes poses significant health risk in humans as a result of utilization of fresh or insufficiently baked seafood. Sea foods generally holds infectious agents as infective germs, which are present in the marine environment either naturally or come from human processes (Saad, 2010). In alive and fresh fish, infective microorganisms may be linked along with the gill part, skin, and gut (Dutta et al. 2014).

Despite upsurge of knowledge on fisheries and its associated diseases, food borne diseases are perhaps among the most widely spread health problem which caused lots of economic meltdown. The available evidence clearly indicates that microbial contaminant is one of the major causes of food borne illnesses (Matthews et al. 2017). Nowadays, fish diseases emerging due to the increase in commercial-scale aquaculture operations have become a major limiting factor in aquaculture (Bondad-Reantaso et al., 2005; Kesarcodi -Watson et al., 2008; Subasinghe et al., 2009). Major bacterial pathogens leading to fish diseases are *Aeromonas salmonicida* (Furunculosis), *Yersinia ruckeri* (enteric red mouth disease), *Vibrio anguillarum* (vibriosis), *Aeromonas hydrophila* (minor surface lesions, septicemia, internal bleeding) and *Lactococcus garvieae* (hemorrhagic septicemia and meningoencephalitis) (Öztürk and Altınok, 2014). These diseases are quite common all over the world and cause significant financial deficits in salmon and trout aquaculture (Austin and Austin, 2012). Therefore, the aim of the current study is to isolate and identify some bacterial pathogens from naturally contaminated or infected *Thunnus tonggol*, and *Caranx sem* Omani fish present in Muscat fish market.

Materials and Methods

Laboratory analysis

Fish samples *Thunnus tonggol* (Longtail Tuna), and *Caranx sem* (Blacktip trevally) were picked from the fish marketplace in Muscat, where the fishes were repeatedly captured from the Omani sea. Totally 15 numbers of samples were collected from each type during the period between September 2019 to October 2021. The picked samples were immediately transferred in icebox to the lab and handled within 3hrs, and samples were kept refrigerated (4–8 ˚C).

Sample preparation

A 10 gm of the fish-sample was removed from skin with a sterilized blade. The chopped sections were pulverized into little fragments in a sterile mortar using 10 ml buffer. From the pulverized section, one milliliter portion was determined out and mixed in a dry sterile container with nine milliliters of dH_2O giving a 1:10 dilution. This was done for all fish samples (Obi and Krakowiaka 1983).

Isolation and identification bacteria form fish sample of *T. tonggol*, and *C. sem*

Samples were handled under aseptic conditions in the research facility, A'Sharqiyah University based on the standard microbiological methods. Swabs were inoculated on, mannitol salt agar and nutrient agar (Oxoid) and incubated aerobically at 37 ˚C. Isolated bacterial colonies were identified based on their morphological, physiological and biochemical characteristics. Cultures were subjected to a variety of biochemical tests such as gram staining, motility, indole, MR/VP, and other tests to identify bacterial isolates using Bergey's manual of systematic bacteriology, (Holt et al. 1994). Bacteria such as *A. hydrophila, A. salmonicida, C. freundii, E. piscicida, E. tarda, H. alvei, L. garvieae, S. marcescens, Y. ruckeri, E. coli, K. pneumonia, P. aeruginosa* and *S. aureus*, were isolated from the fish samples.

Identification of bacterial isolates

Identification of the bacterial isolates was carried out using the phenotypic and biochemical characteristics according to Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2016). Primarily, pure cultures were obtained after repeated plating on selected nutrient media. Identification was based on morphological characteristics, motility, Gram-staining reactions, growth on various types of nutrient media and catalase as well as oxidase tests.

Results and Discussion

Fifteen fishes of *Thunnus tonggol*, and 15 *Caranx sem* were examined. The percentage of *Aeromonas* spp. was 19.5% for all examined samples for both species of fish, *E. coli* and *S. aureus* 14.6% each, *P. aeruginosa* 12.2%, *K. pneumonia* 9.8%, *Edwardsiella* spp., 9.7%, *Y. ruckeri* 7.3% and the rest of isolated bacteria including *C. freundii, H. alvei, L. garvieae*, and *S. marcescens* comprise less than 12%. As a total of 41 isolates from both *Thunnus tonggol* (26 isolates) and *Caranx sem* (15 isolates). A two species each of *Aeromonas* and *Edwardsiella* were identified with different numbers and percentage according to morphological characteristics, Gram stain and biochemical test (Table 1).

The isolated bacteria were identified to be gram positive and gram negative (rod and cocci). The gram-positive cocci resemble *Staphylococcus and lactococcus* and the rod shape gram negative bacteria resemble *E. coli, P. aeruginosa, K. pneumonia, C. freundii, H. alvei* and *S. marcescens* (Table 1 and 2). The results revealed that *Staphylococcus aureus* and *Escherichia coli* was the most abundant pathogenic bacteria associated with the sampled fishes in Muscat. The presence of *Staphylococcus aureus* was ascribed to the defilement of the sampled fish by man activities through mishandling and processing (Clucas and Ward, 1996). This indicates that fresh fish with these bacterial pathogens must have been contaminated through improper handling during post-harvest. Similarly, poor handling may led to contaminating fresh fish with man natural flora such as *Staphylococcus aureus*, *Salmonella typhi* (Saad, 2010; Raufu, 2014), and *Eshcherichia coli* which both grow well at 31–37 °C (Mhango et al. 2010). *Staphylococcus aureus* and *Eshcherichia coli* from the skin of *Clarias gariepinus*

indicate improper management and have been implicated in fish-borne diseases of humans (Cho et al., 2004). Ikpi and Offem, (2011), studied mudfish' skin, gut and gill for presence of microbes leading to the identification of *Clarias gariepinus*, *Staphylococcus aureus Eshcherichia coli* and *Pseudomonas fluorescens*. The results from this study propose that the biological quality of the examined fish is unacceptable and present a possible threat to public health (Ikpi and Offem, 2011).

Table 1. Results of biochemical tests of the bacterial species isolated from fish

Bacterial isolate	Coagulase	Oxidase	Indole	Catalase	Lactose	Mannitol	Glucose	Sucrose	Fructose	Motility
Aeromonas hydrophila	-	+	+	+	-	+/-	+	+	-	+
Aeromonas salmonicida	-	+	+	+	+	+	+	+	+	+
Citrobacter freundii	-	-	-	+	+	+	+	+	v	+
Edwardsiella piscicida	-	-	+	+	-	-	+	-	+/-	v
Edwardsiella tarda	-	-	+	+	-	-	+	-	+/-	+
Hafnia alvei	-	-	-	+	-	+	+	-	+/-	v
Lactococcus garvieae	-	-	-	-	-	+	+	+/-	v	-
Serratia marcescens	-	-	-	+	-	+	+	+	v	+
Yersinia ruckeri	-	-	+	+	-	+	+	-	v	+
Escherichia coli	-	-	+	+	+	+	+	+/-	-	+
Klebsiella pneumonia	-	-	+	+	+	v	+	-	v	+
Pseudomonas aeruginosa	-	+	-	+	-	+	+v	-	v	+
Staphylococcus aureus	+	-	-	+	+	+	+	+	+	-

Table 2. Incidences of the bacterial species isolated from diseased fish

Name of Bacteria	Total bacterial number	Frequency (%)
Aeromonas hydrophila	5	12.2%
Aeromonas salmonicida	3	7.3%
Citrobacter freundii	2	4.9%
Edwardsiella piscicida	3	7.3%
Edwardsiella tarda	1	2.4%
Hafnia alvei	1	2.4%
Lactococcus garvieae	1	2.4%
Serratia marcescens	1	2.4%
Yersinia ruckeri	3	7.3%
Escherichia coli	6	14.6%
Klebsiella pneumonia	4	9.8%
Pseudomonas aeruginosa	5	12.2%
Staphylococcus aureus	6	14.6%

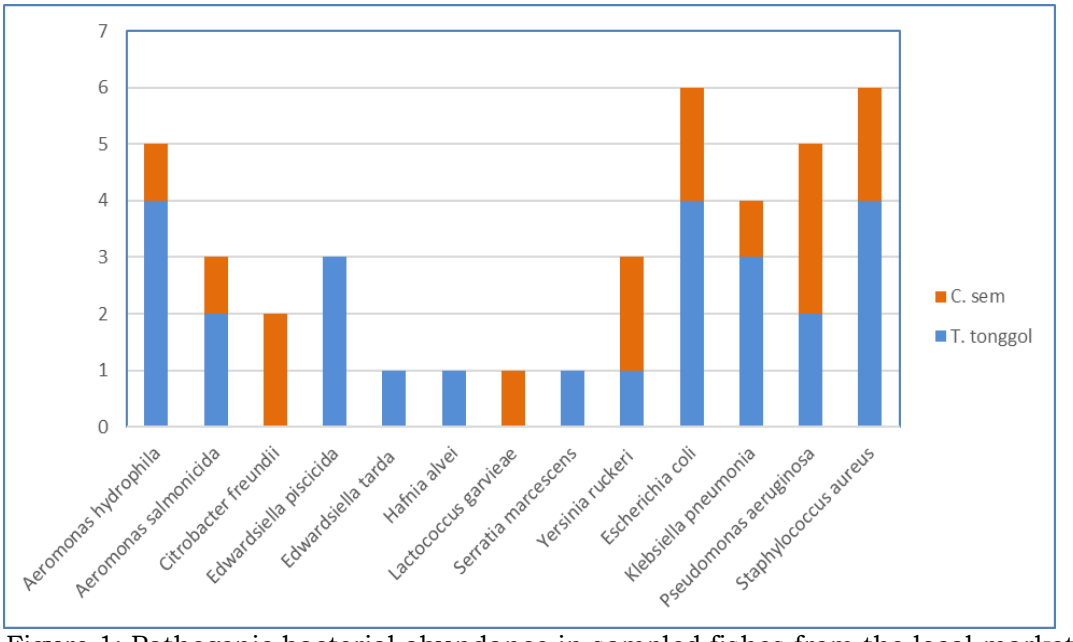

Figure 1: Pathogenic bacterial abundance in sampled fishes from the local market

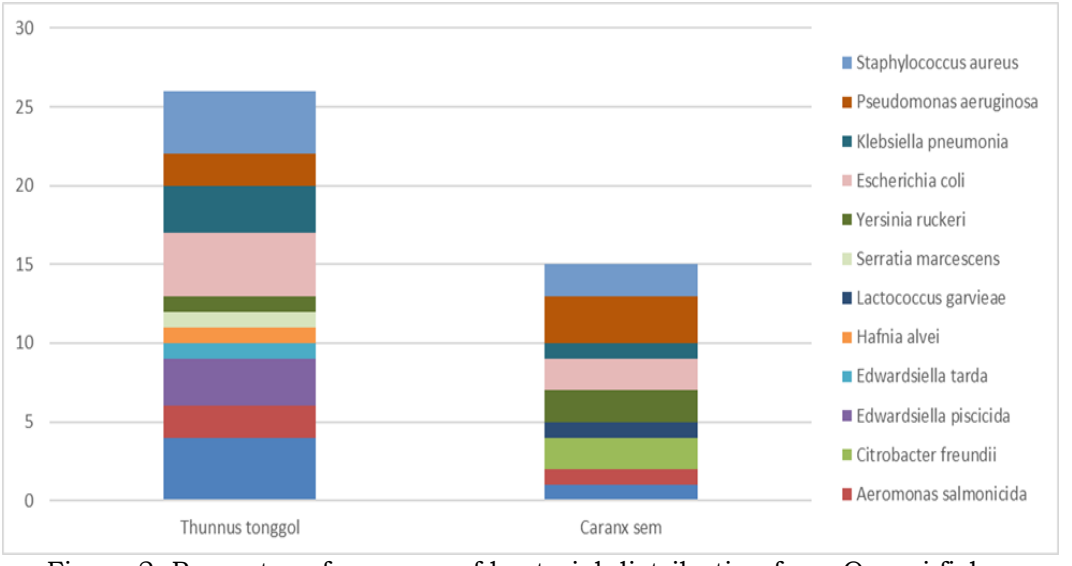

Figure 2: Percentage frequency of bacterial distribution from Omani fishes

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