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Prevalence of *Vibrio Parahaemolyticus* in Various Seafood Consumed in North Cyprus

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BACKGROUND/AIMS
This study was conducted to determine the prevalence of *Vibrio parahaemolyticus* in various finfish and shrimps consumed in North Cyprus.

MATERIAL and METHODS
A total of 150 seafood samples were collected from major seafood retail markets and sea coasts of Famagusta, Kyrenia, Nicosia, and Morphou regions, and they were examined for the prevalence of *V. parahaemolyticus*. After the removal of the hard shell, the gills and intestines of each fish and whole shrimps were separately enriched and isolated on Thiosulfate-citrate-bile salts-sucrose (TCBS) agar. Suspect isolates were selected for biochemical identification and confirmation by the BD Phoenix Instrument.

RESULTS
None of the samples contained *V. parahaemolyticus*. However, *Photobacterium damselae* and *Providencia rettgeri* were detected in 20% of sea bass and sea bream fish species from Kyrenia and Morphou regions. The concentrations of these pathogenic bacteria were >105 cfu/mL (minimum infective dose).

CONCLUSION
V. parahaemolyticus were not detected in any of the examined fish samples taken from different regions of the TRNC. However, seafood consumed in North Cyprus might be a source of other bacterial pathogens, such as *P. damselae* (formerly *V. damsela*) and *P. rettgeri*, because the concentrations of these bacteria in the intestines of sea bass and sea bream fishes from Kyrenia and Morphou regions, respectively, were found to be >105 cfu/mL (minimum infective dose). It is highly recommended to investigate the occurrence and epidemiology of *P. damselae* and *P. rettgeri* in various seafood products because they are pathogenic in both humans and animals.

Keywords: *Vibrio parahaemolyticus*, prevalence, seafood, North Cyprus

INTRODUCTION

Vibrio parahaemolyticus is a human enteropathogenic, sucrose non-fermenting, facultative, and halophilic bacterium that is widely distributed in both marine and estuarine habitats and is present in seafood harvested from aquatic environments worldwide (1, 2). This marine-based enteropathogenic bacterium is responsible for most of the seafood-borne bacterial illnesses leading to gastrointestinal problems such as nausea, vomiting, abdominal cramps and watery or bloody diarrhea sometimes accompanied with fever (3).

The debilitating effects of *V. parahaemolyticus* are because of the presence of virulence genes (t^{dh} and t^{rh}), type III secretion systems (T3SSI and T3SS2), clonal serotypes (O3:K6 and its serovariants), and extracellular proteases (4-9). Antibiotic treatment is not usually needed for *V. parahaemolytiticus* poisoning, however, in cases with prolonged diarrhoea, antibiotic therapy can be given.

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TABLE I. Sampling regions in TRNC and number of primary samples taken

Region	Fish species	Number of primary samples
Famagusta	Sea bass	5
	Sea bream	5
	Shrimp	10
	Catch of the day: Mackerel	5
	Catch of the day: Marbled spinefoot	5
Kyrenia	Sea bass	5
	Sea bream	5
	Shrimp	20
	Catch of the day: Blue whiting	5
Nicosia	Sea bass	5
	Sea bream	5
	Shrimp	30
Morphou	Sea bass	5
	Sea bream	5
	Shrimp	30
	Catch of the day: Mackerel	5

A significant number of individuals worldwide depend on seafood as a primary source of valuable nutrients, particularly protein, poly unsaturated fatty acids, vitamins, and minerals (10). Virtually, the nutritional value of seafood has led to its worldwide acceptance and excessive consumption. In recent years, the world consumption per capita of marine and aquaculture fishery products has reached over 20 kg a year (11). Accordingly, European Market Observatory for Fisheries and Aquaculture Products (EUMOFA) reported similar trends in the consumption per capita of 23.9 kg in the EU member states (12).

According to a report released by the Food and Agriculture Organization of the United Nations (FAO), Mediterranean seafood production has increased in the previous decades because of the large production of sea bream and sea bass (13). Concurrently, an epidemiological report has shown that among seafood-borne pathogens, *V. parahaemolyticus* present a significant threat, and shrimp and finfish are high on the list of the most contaminated seafood (2, 14). Surveying, monitoring, and detecting pathogens in foods are the most important approaches to reduce, control, or prevent foodborne bacterial infections (15). Bacterial infections mostly because of the consumption of fish and shellfish have been attributed to pathogenic *Vibrios* (16).

In the Turkish Republic of Northern Cyprus (TRNC), like other Mediterranean countries, the most important finfish consumed are sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax*). Among shellfish, shrimp is the most widely consumed. To the best of our knowledge, the occurrence of *V. parahaemolyticus* in the seafood consumed in the TRNC has never been investigated. Hence, the present study aimed to investigate the occurrence of *V. parahaemolyticus* in retail sea bream (*S. aurata* L.), European sea bass (*D. labrax*), and shrimps. The various seafood caught along the coast of the Mediterranean Sea in Famagusta and Kyrenia have also been included in the present study.

MATERIAL and METHODS

Seafood samples in this study include European sea bass (*D. labrax*), gilt-head sea bream (*S. aurata* L.), blue whiting (*Micromesistius poutassou*), marbled spinefoot (*Siganus rivulatus*), mackerel (*Scomber scombrus*), and shrimp. These fish varieties were selected because they are widely consumed and are available throughout the year. Seafood samples were taken from major seafood outlets of Famagusta, Kyrenia, Nicosia, and Morphou regions and also directly from the coasts and/or bays of the Famagusta and Kyrenia regions (Table I). Five randomly selected fishes and 10 randomly selected shrimps (totaling approximately 1 kg) were taken from each shop's daily sold during Summer and early-Fall seasons of 2016. These representative samples were drawn in accordance with standardized procedures for fresh seafood sampling (17, 18). No ethical committee was consulted because no live animal experiment was conducted in this study, consequently no ethical information can be given.

The isolation and identification of *V. parahaemolyticus* by a conventional culture technique was done in accordance with Food and Drug Administration/Bacteriological Analyses Manual (FDA/BAM) (19). Seemingly, apathogenic and pathogenic bacteria live on the skin, in the gills, and in the intestines of fish. Therefore, the gills and intestines from each fish sample were removed and then separately homogenized in 225 mLof alkaline peptone water (APW) with 3% NaCl for 1 min.

Shrimp samples were thoroughly washed under running water and shucked for sampling according to Cook et al. (20). Samples were comminuted to get a puree representing the whole sample location. A total of 25g puree was taken and then homogenized in 225 mL of APW. The fish and shrimp homogenates were transferred into sterile polythene stomacher bags and incubated in an incubator (Thermo Scientific, Massachusetts, USA) at 37°C for 24h. After incubation (24 h), 1 mL of each homogenate was taken aseptically using a sterile wooden cotton applicator

TABLE 2. Incidence of bacterial pathogens in seafood consumed in the TRNC

Region	Seafood	Number of samples positive/ number of samples examined	Identified Pathogen	Concentration of pathogen (cfu/mL)
Famagusta	Sea Bass	0/5		
	Sea Bream	0/5		
	Shrimp	0/10		
	Mackerel	0/5		
	Marbled spinefoot	0/5		
Kyrenia	Sea Bass	1/5	*Providencia rettgeri*	$>10^5$
	Sea Bream	0/5		
	Shrimp	0/20		
	Blue whiting	0/5		
Nicosia	Sea Bass	0/5		
	Sea Bream	0/5		
	Shrimp	0/30		
Morphou	Sea Bass	0/5		
	Shrimp	0/30		
	Sea Bream	1/5	*Photobacterium damselae*	$>10^5$
	Mackerel	0/5		

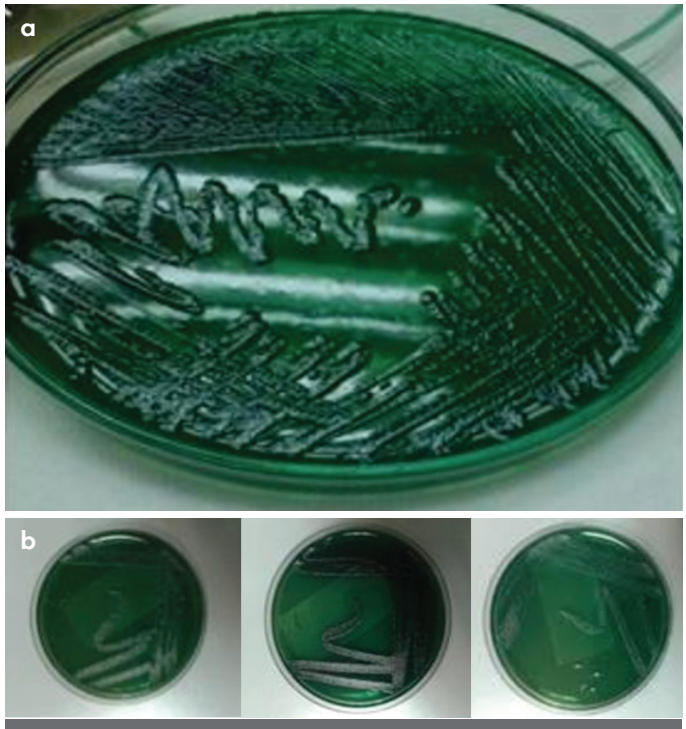

FIGURE 1. a, b. *Vibrio parahaemolyticus* American type culture collection (ATCC) 17802 growth on: thiosulfate-citrate-bile salts-sucrose (TCBS) agar plate (a). Suspected TCBS agar plates (b)

stick and streaked onto sterile surface thiosulfate-citrate-bile salts-sucrose (TCBS; Liofilchem s.r.l., Teramo, Italy) agar plates. The plates were then incubated at 37°C for 24h.

Following plate incubation, TCBS plates were checked for suspect colonies that were sucrose non-fermenting with a green or bluish-green color and a dark blue or green center approximately 3–5 mm long indicating the presence of *V. parahaemolyticus*. Such colonies were carefully selected. The suspect colonies were purified and further characterized by performing catalase and gram-staining tests. Suspect isolates that were positive for catalase and that were gram-negative-stained were selected for biochemical identification and confirmation. *Vibrio parahaemolyticus* ATCC 17802 was used as control (Figure 1a). Suspect isolates were screened by automated identification and antimicrobial testing (BD Phoenix, Franklin Lakes, USA).

RESULTS

V. parahaemolyticus was not detected in any of the examined fish and shrimp samples, but other gram-negative bacteria were detected in the intestines of sea bass from Kyrenia and sea bream from Morphou regions. Following a series of biochemical tests and by the BD Phoenix Identification Instrument, three bacterial species, including *Photobacterium damselae* (formerly *V. damsela*), *Providencia rettgeri*, and *Pseudomonas fluorescens*, were confirmed. Two of these bacteria, namely, *P. damsalae* and *P. rettgeri*, are pathogenic in both humans and animals. Results for seafood species, locations, and pathogens are presented in Table 2 and suspected bacterial colonies on TCBS agar in Figure 1b.

DISCUSSION

Surveying, monitoring, and detecting pathogens in foods are the most important approaches to reduce, control, or prevent foodborne bacterial infections (15). Bacterial infections mostly because of the consumption of fish and shellfish have been attributed to pathogenic *Vibrios* (16).

Fortunately, *V. parahaemolyticus* was not found in any seafood species sampled in our study, although a study from the United States reported elevations in the number of Vibrio infections associated with seafood (16). In Europe, *V. parahaemolyticus* has been considered to be an emerging foodborne pathogen

responsible for most of the recent sporadic and epidemic seafood-borne infections (21, 22). According to Abd-Elghany and Sallam (23), 120 shellfish samples (40 each of shrimp, crab, and cockle) were collected from different fish shops in Mansoura, Egypt and tested for the presence of potentially pathogenic strains of *V. parahaemolyticus*. The conventional technique as shown by biochemical means showed that 40 (33.3%) out of 120 samples were positive for *V. parahaemolyticus*. *V. parahaemolyticus* was found in 4.0% of the winter samples, 13.3% of the spring samples, 18.6% of the summer samples, and 8% of the autumn samples. A significant proportion of shrimps marketed and consumed in Morocco caught in the coastal region of the city of Agadir contained *V. parahaemolyticus* (24). The absence of *V. parahaemolyticus* in the shrimps in our study may be because of seasonal variations. However, the results of this study are in agreement with those of a previous study conducted in some European countries where fish samples sourced from France and Great Britain did not contain *V. parahaemolyticus* (25).

Nonetheless, other aquatic bacterial pathogens, such as *P. damselae* and *P. rettgeri*, were found in our fish samples. *P. damselae* is a pathogen for several species of fish and shellfish. In humans, this bacterium can cause a wide range of infections that may result in necrotizing fasciitis usually with severe clinical consequences. For over two decades, *P. damselae* has become a threat for several species as well as humans worldwide (26-28). *P. rettgeri* is one of the major causes of diarrhea in humans. It is also a major source of tetrodotoxin (a potential neurotoxin), predominantly in some Asian countries and recently in Europe, and its abundance in various fish species is widely increasing (29-31).

These bacteria could be isolated because they are also sugar non-fermenting and gram-negative like *V. parahaemolyticus* and share similar growing conditions in the sea. The incidence of these pathogens could be an alert to maximum exposure to multiple microbial pathogens from seafood (32).

In conclusion, *V. parahaemolyticus* was not detected in any of the examined fish samples taken from different regions of the TRNC. However, seafood consumed in might be a source of other bacterial pathogens, such as *P. damselae* and *P. rettgeri* species, because the concentrations of these bacteria were found to be >10^5 cfu/mL (minimum infective dose) in the intestines of sea bass and sea bream fishes from Kyrenia and Morphou regions, respectively. It is highly recommended to investigate the occurrence and epidemiology of these species in various seafood products because they are pathogenic in both humans and animals.

Ethics Committee Approval: No ethical committee was consulted because no live animal experiment was conducted in this study, consequently no ethical information can be given.

Informed Consent: N/A

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Conflict of Interest: The authors have no conflicts of interest to declare.

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