Microbiological quality of marketed fresh and frozen seafood caught off the Adriatic coast of Croatia

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ABSTRACT: Fresh and frozen seafood products (fish, shellfish, crustaceans, molluscs) in wide use in Croatia and typical of the Mediterranean diet, were examined for the presence of microbiological contamination through the winter and summer seasons. Total bacterial counts of aerobic mesophilic bacteria (AB), aerobic psychrophilic bacteria (AP), *Salmonella* spp., *Enterobacteriaceae*, *Escherichia coli*, *Staphylococcus aureus*, sulphite-reducing clostridia (SRC), *Listeria monocytogenes*, *Vibrio cholerae* and *V. parahaemolyticus* were measured. The microbiological quality of individual samples varied widely between animal species and also between winter/summer seasons regarding total counts of aerobic mesophilic and psychrophilic bacteria. The poorest quality was for (both summer and winter) fish samples, where 66.6 % of fresh and frozen fish were found unacceptable by Croatian standards. The overall prevalence of *V. parahaemolyticus* was 5%. Its recovery rate was higher in fresh/frozen shellfish in both seasons than in other specimens or other storage/season conditions. Fresh crustaceans sampled in winter demonstrated significantly higher aerobic mesophilic counts than frozen ones. Unacceptable *Enterobacteriaceae* levels were obtained in 40% of the fresh fish summer samples. The results of this survey constitute an indicator of bacteriological contamination of a variety of seafood. The findings could serve as a basis for future testing of seafood, and possibly as a template for developing a regional/Mediterranean testing scheme on the microbial contamination of seafood in order to establish data with comparative epidemiological and statistical values.

Keywords: enterobacteria; vibrios; microbiological contamination

The Adriatic Sea has a relatively moderate fish stock but a large number of fish species. Registered catches of sea fish and other sea organisms amount to over 50 000 tonnes annually. Small pelagic fish comprise the majority of the total catch, while the annual production of mariculture is about one fifth of the catch number (Croatian Chamber of Economy, 2004, 2009). Seafood, although an important element of Mediterranean diets, plays a significant role in causing food borne diseases. Fresh seafood is a highly perishable product and spoilage developing in aerobically stored fish typically consists of Gram-negative psychrophilic non-fermenting rods. Thus, under aerobic ice storage, the flora is composed almost exclusively of *Pseudomonas* spp. and *Shewanella putrefaciens* (Chouliara et al., 2004). The *Enterobacteriaceae* count is considered as another index of fish quality because it is related to storage in ice, washing and evisceration (Zambuchini et al., 2008). A monitoring of these microorganisms has been suggested as a measure of fish quality. Also, risk management decisions should take into account the whole food chain from primary production to consumption, and should

be implemented in the context of appropriate food safety infrastructures, for instance regulatory enforcement, food product tracing and traceability systems. In the fish processing chain managing risks should be based on scientific knowledge of the microbiological hazards and the understanding of the primary production, processing and manufacturing technologies and handling during food preparation, storage and transport, retail and catering (Reilly, 2006).

Seafood products harvested from contaminated waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio* spp. (Baffone et al., 2000). Consumption of raw or undercooked seafood, particularly shellfish, contaminated with *V. parahaemolyticus* may lead to development of acute gastroenteritis characterized by diarrhoea, headache, vomiting, nausea, abdominal cramps and low fever. This bacterium is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States and an important seafood-borne pathogen throughout the world (Farmer et al., 2003; Su and Liu, 2007). In contrast to Asian countries, *V. parahaemolyticus* infections are rarely reported in European countries (Feldhusen, 2000). However, sporadic outbreaks have been reported in some Mediterranean countries. In Croatia, *Salmonella* spp. is responsible for the majority of food-borne illnesses and constitute the primary microbial pathogens. (Mulic et al., 2004).

In the present study, fresh and frozen seafood were randomly selected and collected from the biggest inland market in Croatia in warm (July, August) and cold (December, January) seasons of the year and were analyzed for their microbiological quality. Although constant control of seafood is provided for by veterinary inspection under the supervision of state institutions, the main purpose of this work was to investigate the presence and the level of aerobic mesophilic bacteria (AB), aerobic psychrophilic bacteria (AP), *Salmonella* spp., *Enterobacteriaceae*, *Escherichia coli*, *Staphylococcus aureus*, sulphite-reducing clostridia (SRC), *Listeria monocytogenes*, *Vibrio cholerae* and *V. parahaemolyticus* in seafood presented for direct human consumption and evaluate their possible seasonal variations. Most published work regarding microbial contamination of seafood generally concentrates on a limited spectra of bacteria, mostly vibrios (particularly *V. parahaemolyticus*) and coliforms, and this study attempts to provide a broader insight into the microbiological quality of presented seafood.

MATERIAL AND METHODS

Sampling

Fresh and frozen marine **fish** [sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), sprat (*Sprattus sprattus*), pilchard (*Sardina pilchardus*), red scorpionfish (*Scorpaena scrofa*), bluefin tuna (*Thunnus thynnus*), hake (*Merluccius merluccius*), striped red mullet (*Mullus surmuletus*)]; **shellfish** [Mediterranean mussel (*Mytilus galloprovincialis*), scallop (*Pecten jacobaeus*), Noah's ark (*Arca noae*), warty venus (*Venus verrucosa*), cockle (*Cerastoderma glaucum*)]; **crustaceans** [Norway lobster (*Nephrops norvegicus*), red shrimp (*Aristeus antennatus*)]; and **molluscs-cephalopods** [European squid (*Loligo vulgaris*), squid (*Todarodes sagittatus*)] were collected from a major inland market in Croatia.

Fresh seafood were caught off the eastern coast of Croatia in the Adriatic Sea by local fishermen, then transported overnight on ice to the market in the continental part of the country and were presented for customers early in the morning, when they were randomly selected and sampled. Samples were collected by an official inspector in summer and in winter as specimens that were representative for that market's offer of the day. A total of 240 samples were used in this study. Each sampling (summer (July, August)/winter (December, January); fresh/frozen) comprised of 15 fish samples, 15 crustacean samples (each crustacean sample comprised about five individuals from the same batch), 15 shellfish samples (each shellfish sample comprised about 10 individuals from the same batch), 15 mollusc samples. A total of 60 fresh and 60 frozen samples were collected in both seasons. Frozen samples represented a typical deep-freeze market supply.

Sample preparation

Following their collection, samples were immediately delivered to the laboratory on ice and under hygienic conditions. After rinsing the shellfish in sterile distilled water to remove any debris on the shell, samples were further prepared for bacterial isolation by cutting out, with sterile tools, 10 and

25 g of interior flesh content of shellfish and crustaceans, and fish and molluscs, respectively.

Bacteriological analysis

The homogenate samples were inoculated on general purpose media, standard plate count media, media for fastidious organisms and media for the selective isolation of bacteria. After the inoculation procedures, the bacterial colonies were isolated from samples. The isolated colonies were subjected to bacteriological characterization procedures. First the putative colonies were determined on selective media, while further identification was conducted by using morphological, physiological and biochemical tests. Total bacterial count of aerobic mesophilic bacteria (AB), aerobic psychrophilic bacteria (AP), *Salmonella* spp., *Enterobacteriaceae*, *Escherichia coli*, *Staphylococcus aureus*, sulphite-reducing clostridia (SRC), *Listeria monocytogenes*, *Vibrio cholerae* and *V. parahaemolyticus* tests were performed. All media used were from Oxoid Ltd, UK.

Total viable count, methods for *Salmonella* spp., *E. coli*, *Enterobacteriaceae*, *S. aureus*, SRC, and *L. monocytogenes*

After homogenization, samples for AB and AP colony count were serially diluted and plated using the pour-overlay method on solid medium using Iron agar (Agar Lyngby-IA, Oxoid) aerobically incubated for three days at 30°C (AB) and 21°C (AP) and counted as colony forming units (CFU/g) according to the Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique (ISO 4833, 2003).

Salmonella spp. were determined according to the Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of *Salmonella* spp. (ISO 6579, 2002), which specifies a horizontal method for the detection of *Salmonella*, including *S. typhi* and *S. paratyphi.* Pre-enrichment was conducted from samples diluted in 225 ml buffered peptone water incubated at 37°C for 18 hours. Secondary selective enrichment was performed in Rappaport-Vassiliadis peptone broth (41°C for 24 h) and Muller-Kaufmann tetrathionate broth with Novobiocin (37°C for 24 h), and plating on XLD agar and Rambach agar, XLT-4 agar (37°C for 24 h).

E. coli were determined according to the Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive *E. coli* – Part 2: Colony count technique at 44°C for 24 h using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide (ISO 16649, 2001). Tryptone Bile X-glucuronide Agar (TBX/TBGA) based on the formulation of Tryptone Bile Agar with the addition of the X-glucuronide chromogen was used. On this medium *E. coli* appeared as distinctive green/blue colonies.

Enterobacteria were determined according to the Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count method (ISO 21528-2, 2004) which specifies a method, without pre-enrichment, for the enumeration of *Enterobacteriaceae*. Enumeration was carried out by counting colonies on a solid medium after incubation at 37°C for 24 hours. This technique is recommended when the number of colonies sought is expected to be more than 100 per ml or per gram of the test sample. Violet Red Bile Glucose Agar was used for the detection and enumeration of *Enterobacteriaceae*.

S. aureus were determined according to the Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphyloccoci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium (ISO 6888-1, 1999 + Amd 1, 2003).

SRC were determined according to the Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of sulphite-reducing bacteria growing under anaerobic conditions (ISO 15213, 2004). The first step in the analysis of sulphite-reducing *Clostridium* spores was heat treatment of the tubes containing the first decimal dilution (in buffered peptone water) in a thermostatically controlled water bath at 75°C for 20 min, to eliminate vegetative cells. Iron Sulphite Agar was inoculated and anaerobically incubated at 37°C for 24–48 hours.

L. monocytogenes were determined according to the Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method (ISO 11290-1, 1996) on *Listeria* Enrichment Broth Base Fraser, which is an appropriate medium for preparing Fraser by adding the respective supplements. Pre-enrichment was conducted in Half Fraser Broth for 24 h at 30°C. After incubation, the primary and secondary enrichment

tubes were inoculated onto Oxford and ALOA agar (Agar Listeria selon Ottaviani and Agosti) and incubated at 37°C for 48 hours.

Method for *Vibrio* identification

Vibrios were detected according to the Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 1: Detection of *V. parahaemolyticus* and *V. cholerae* (ISO/TS 21872-1, 2007), which specifies a horizontal method for the detection of these two main pathogenic *Vibrio* species causing intestinal illness in humans. Samples were homogenized in 225 ml of 3% NaCl containing alkali peptone solution. The homogenate was further incubated for 6 h at 37°C (for frozen seafood) and at 22°C (for fresh seafood). After incubation, 1 ml of alkaline saline peptone water was placed in a tube containing 10 ml of alkaline saline peptone water, and incubated for 18 h at 41.5°C before being inoculated onto Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) and incubated for 24 h at 37°C. To facilitate identification of suspect *Vibrio* isolates, the commercial biochemical test kit API 20 NE (BioMérieux SA, Marcy l'Etoile, France) was used, and the identification was obtained by using the identification software "apiweb".

Statistical analysis

A test of significance of observed differences in levels of retrieved bacteria and seasonal/seafood species parameters was conducted using a one-way analysis of variance (ANOVA) and ANOVA on ranks. Also, the Mann-Whitney Rank Sum test was used. The *t*-test was used to establish if the means of two different groups under comparison were

Table 1. Total number of aerobic mesophilic bacteria (AB) in summer/winter sampling for fish/crustaceans/shellfish/molluscs. Counts represent colony forming units per gram of sample. According to the Croatian microbiological standards for foods, acceptable limits for fresh/frozen fish are 10^3 CFU/g, for fresh/frozen shellfish and molluscs 10^5 CFU/g, while for fresh/frozen crustaceans the figure is 10^4 CFU/g

Sample type	Number of samples with count < 10^3	Number of samples with count between 10^3–10^5	Number of samples with count > 10^5	Percentage of samples unacceptable
Summer				
Frozen fish	5	10	0	66.6
Fresh fish	5	6	4	66.6
Frozen crustaceans	3	12	0	40
Fresh crustaceans	3	12	0	40
Frozen shellfish	2	13	0	0
Fresh shellfish	3	12	0	0
Frozen molluscs	1	10	4	26.6
Fresh molluscs	1	12	2	13.3
Winter				
Frozen fish	5	10	0	66.6
Fresh fish	5	10	0	66.6
Frozen crustaceans	4	11	0	40
Fresh crustaceans	0	15	0	80
Frozen shellfish	2	13	0	0
Fresh shellfish	0	13	2	0
Frozen molluscs	1	11	3	20
Fresh molluscs	1	12	2	6.6

significantly different in the normally distributed population from which the samples were drawn. $P < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Total counts of aerobic mesophilic bacteria (AB) and total counts of aerobic psychrophilic bacteria (AP) in summer/winter samplings are presented in Tables 1 and 2, respectively, as percentages of samples designated according to the bacterial counts for different animal groups and fresh-frozen status. The microbiological quality of individual samples varied widely between the animal species and between the winter and summer seasons. The poorest quality, according to the Croatian microbiological standards for foods which allows AB limits for fresh/frozen fish at 10^3 CFU, was that of summer and winter fish samples. Sixty-six point six percent of fresh and frozen fish were unacceptable and as many as 26.6% fresh summer fish yielded over 10^5 CFU. Investigated fresh/frozen crustaceans have AB limits at 10^4 CFU, thus rendering 40% of summer fresh and frozen crustaceans, as well as 80% of winter fresh crustaceans unacceptable. Interestingly, both summer and winter samplings for both fresh and frozen shellfish had acceptable AB levels. However, median values for AB counts of the fresh shellfish sampled in summer were significantly higher than AB counts of the fresh shellfish sampled in winter ($P = 0.031$). Over 26.6% of summer frozen molluscs and 13.3% of fresh molluscs were over the allowed limits for molluscs (10^5 CFU), with just a slightly better situation in winter, 20.0% frozen and 6.6% fresh molluscs, respectively. Fresh crustaceans sampled in winter had significantly higher AB counts than frozen ones ($P = 0.044$). As visible in Table 2, AP counts were significantly higher in fresh fish than in frozen fish sampled in summer ($P = 0.026$). A significant difference ($P < 0.05$) was also demonstrated between summer fresh molluscs and frozen fish, as well as between winter fresh shellfish and

Table 2. Total number of aerobic psychrophilic bacteria in summer/winter sampling for fish/crustaceans/shellfish/molluscs. Counts represent colony forming units per gram of sample

Sample type	Number of samples with count < 10^2	Number of samples with count between 10^2–10^3	Number of samples with count > 10^3
Summer			
Frozen fish	0	15	0
Fresh fish	1	8	6
Frozen crustaceans	0	12	3
Fresh crustaceans	0	12	3
Frozen shellfish	0	14	1
Fresh shellfish	0	1	14
Frozen molluscs	0	12	3
Fresh molluscs	0	10	5
Winter			
Frozen fish	0	15	0
Fresh fish	0	15	0
Frozen crustaceans	0	12	3
Fresh crustaceans	0	12	3
Frozen shellfish	0	14	1
Fresh shellfish	0	8	7
Frozen molluscs	0	12	3
Fresh molluscs	0	13	2

all other animal groups (fresh or frozen) sampled in winter. When comparing summer and winter data, AP counts were significantly higher in fresh fish sampled in summer than in winter ($P = 0.027$); as well as in fresh shellfish sampled in winter over those sampled in summer ($P = 0.044$).

No *Listeria monocytogenes, Salmonella* nor *V. cholerae* were isolated. In Italy, however, a two-year survey demonstrated a level of *Salmonella* in seafood of 0.5%, while *L. monocytogenes* was present in 6.5% of fish (Busani et al., 2005).

In this study *Staphylococcus aureus* and SRC were present at < 100 CFU/g in all samples. Of the 240 samples analyzed, one fresh winter shellfish sample (0.41%) showed a level of *E. coli* CFU exceeding the given guideline of $< 10^2$ per g by four-fold, and therefore did not comply with the current legislation. That sample also had high levels of *Enterobacteriaceae*. No correlation between *E. coli* contamination and the presence of potentially pathogenic vibrios was found, which is in agreement with other findings relating to shellfish (Normanno et al., 2006; Lhafi and Kuhne, 2007). *Enterobacteriaceae* levels permitted by Croatian microbiological standards for food and food products are $< 10^2$ CFU/g for fresh and frozen fish, but the assay is not required for fresh and frozen shellfish, molluscs and crustaceans. Unacceptable levels were obtained in 40% of the summer fresh fish samples (Table 3). The poorest quality with regard to high counts of *Enterobacteriaceae* was found for fresh summer molluscs and fish, respectively, with up to 3×10^3 CFU/g. Over 26% of summer frozen molluscs yielded up to 10^3 CFU/g, which does not correlate with the observations of Vaz-Pirez et al. (2008) where counts of *Enterobacteriaceae* for cuttlefish molluscs (*Sepia officinalis*) and broadtail shortfin squid (*Illex coindetii*) stored in ice were negligible. They buttress their results by proving that due to very rapid enzymatic action only a restricted number of bacteria are able to penetrate in fish muscle during storage in ice.

Table 3. Total *Enterobacteriaceae* in summer/winter sampling for fish/crustaceans/shellfish/molluscs. Counts represent colony forming units per gram of sample. According to the Croatian microbiological standards for foods, tolerated limits for fresh/frozen fish are 10^2 CFU/g, while the assay is not required for fresh/frozen shellfish, molluscs and crustaceans

Sample type	Number of samples with count $< 10^2$	Number of samples with count between 10^2–10^3	Number of samples with count $> 10^3$	Percentage of samples unacceptable
Summer				
Frozen fish	15	0	0	0
Fresh fish	9	4	2	40
Frozen crustaceans	15	0	0	N/A
Fresh crustaceans	12	3	0	N/A
Frozen shellfish	15	0	0	N/A
Fresh shellfish	15	0	0	N/A
Frozen molluscs	11	4	0	N/A
Fresh molluscs	9	3	3	N/A
Winter				
Frozen fish	15	0	0	0
Fresh fish	15	0	0	0
Frozen crustaceans	12	3	0	N/A
Fresh crustaceans	15	0	0	N/A
Frozen shellfish	15	0	0	N/A
Fresh shellfish	14	1	0	N/A
Frozen molluscs	12	3	0	N/A
Fresh molluscs	11	4	0	N/A

Table 4. Prevalence of *V. parahaemolyticus* (number of positive samples identified by API 20 NE/prevalence of *Enterobacteriaceae*; number of positive samples with over 100 CFU). Each sampling comprised of 15 fish, crustaceans, shellfish, molluscs samples, respectively. Total number of samples n = 240

	Fish	Crustaceans	Shellfish	Molluscs
Fresh/summer	3/6	0/3	2/0	0/6
Frozen/summer	0/0	0/4	3/0	0/4
Fresh/winter	0/0	0/0	2/1	0/4
Frozen/winter	0/0	0/3	2/0	0/3
Total	3/6	0/10	9/1	0/17

The overall prevalence of *V. parahaemolyticus* was 5% (a total of 12 were identified in this study). Of those, three were retrieved from fish: the prevalence of the pathogen in fish under investigation was 5%, while nine were isolated from shellfish: the prevalence of the pathogen in shellfish under investigation was 15%. The recovery rate was higher in fresh/frozen shellfish in both seasons than in other specimens or under other storage/seasonal conditions, except for fresh fish sampled in summer. The recovery of *V. parahaemolyticus* was more pronounced in fresh species without regard to the season (Table 4). Biochemical characteristics of 12 isolates of bacteria grown on the API 20 NE strip and identified as *V. parahaemolyticus* after 48 h incubation were designated as "Excellent identification" (six isolates, profile number 7077644), "Very good identification" (three isolates, profile number 7077645), or "Good identification" (three isolates, profile number 7075645). The degree of *V. parahaemolyticus* contamination in raw shellfish is known to relate to water temperatures. For example, detection of *V. parahaemolyticus* is more likely in oysters harvested in the spring and the summer than those caught in winter. Although the density of *V. parahaemolyticus* in contaminated oysters is usually lower than 10^3 CFU/g at harvest (Kaysner and DePaola, 2000), it could exceed 10^3 CFU/g in oysters harvested from warmer seawater (DePaola et al., 2000) and the organism can multiply rapidly in oysters upon exposure to elevated temperatures (Su and Liu, 2007). Parveen et al. (2008) reported that the levels of *V. parahaemolyticus* isolated from oysters (*Crassostrea virginica*) were related to water temperature and were not detected when water temperature was less than 9°C. The ubiquitous nature of *Vibrio* species in marine and estuarine environments makes it impossible to obtain seafood free of these bacteria. The significance for public health is dependent on the health status of the consumer as well as on the concentration and on the virulence of the pathogen (Lhafi and Kuhne, 2007).

According to the Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk for human health. The safety of foodstuffs is mainly ensured by a preventive approach, such as implementation of good hygiene practice and application of procedures based on hazard analysis and critical control point (HACCP) principles. Microbiological criteria can be used in validation and verification of HACCP procedures and other hygiene control measures. Since seafood is traditionally a popular part of the diet in Croatia, monitoring the safety of seafood is crucial. In previous decades annual reports have demonstrated an increase in the occurrence of *Vibrio* spp. in Croatian seafood over the seasons (Jaksic et al., 1990). Recent surveys have failed to establish any significant pattern in the occurrence of specific *Vibrio* species in samples of sea fish, shrimps or bivalves harvested from the Adriatic Sea, or their presence depending on the source of samples (Jaksic et al., 2002).

A significant portion of the samples collected for this study were contaminated with enterobacteria. The reason for this enterobacterial contamination could be related to errors and omissions in handling seafood, including cooling of freshly caught seafood products with contaminated seawater, the presence of illicit wastewater disposal in waters destined for the farming of molluscs and the habit of harvesting molluscs in uncontrolled areas as explained by Baffone et al. (2000). Falcao et al. (2002) have provided evidence that the ice used to refrigerate seafood may be contaminated with pathogenic microorganisms and become a vehicle

for human infection, as they discovered the presence of high numbers of coliforms, heterotrophic indicator microorganisms and pathogenic strains in ice used for chilling fish and other seafood; therefore, some of the contamination detected in the current study could be due to the ice used for chilling purposes.

CONCLUSION

The results of this investigation constitute an indicator of bacteriological contamination of a variety of seafood. Overall microbiological quality of fresh/frozen species in different seasons was acceptable. The seafood with the largest number of unsatisfactory rates of indicators and pathogens were molluscs. The largest summer/winter discrepancy regarding bacterial counts was recorded for *Enterobacteriaceae*, where especially fresh fish yielded unacceptable levels in summer. The hazard associated with marketed seafood, determined as the presence of specific bacterial pathogens and indicators, was relatively low, meaning that marketed seafood in Croatia constitutes a minor threat to consumers. Since Croatian seafood is widely exported to other European countries and since the Adriatic is a summer vacation destination for millions, it is important that its microbiological seafood assessment be available. Also, it is always recommended to inform consumers of the possible health hazards related with the consumption of raw or undercooked seafood, especially shellfish since careful handling of seafood, prevention of cross-contamination at the processing or food preparation are crucial in preventing infections associated with seafood pathogens, and consumer health is not always adequately protected despite the effort and resources invested in seafood microbiological quality research. Our findings could serve as a basis for future testing of seafood, and possibly as a template for developing a regional/Mediterranean testing scheme on the microbial contamination of seafood which should be conducted and published in order to establish data with comparative epidemiological and statistical values.

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