Article

Prevalence of microbial contamination of fresh seafood product sold in Constantine, Algeria

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Abstract

The seafood products are considered as an important element in the Mediterranean diet and play a significant role in the appearance of diseases and food poisoning. Forty samples of seafood products from various provenances of eastern coast of Algeria were randomly collected from several retail markets at the Constantine region. Total bacterial counts of Aerobic Mesophilic bacteria, *Salmonella spp*., total and fecal coliforms, *Staphylococcus aureus*, Sulphite reducing anaerobes bacteria, *Vibrio spp*., were measured. 39 samples (97.5%) of the seafood product analysed, were inappropriate for human consumption, solely one sample 2.5 % of sardines were suitable for human consumption. *Salmonella spp*., was detected in one sample (3% of Sardines), Total and fecal coliforms were detected in 39 samples, *Clostridium spp*. were detected in 5 samples. No strain of *Staphylococcus aureus* and vibrio were detected. The results of this study constitute an indicator of bacteriological contamination and showed that samples markets were contaminated with potential pathogenic microorganisms.

Keywords seafood; microbial contamination; pathogenic microorganisms; hygiene.

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1 Introduction

Fish and seafood constitute an important food component for a large section of world population (Bark et al., 2011; Sakthivel and Fernando, 2012; Trivedi et al., 2012; Ozcan et al., 2013; Varadharajan et al., 2013). Fishery products can also be a source of various food borne diseases, (Darlington and Stone, 2001). It acts as a vehicle for all important species of foodborne pathogens. Environmental conditions play an important role on the pathogens count in fresh fish (Bark et al., 2011). Shellfish may be contaminated with food borne pathogens which are naturally present in aquatic environments, such as *Vibrio spp*. or derived from sewage contaminate water such as *Salmonella* (Ali and Hamza, 2004).

Regarding to the potential public health threat that the shellfish may constitute, the main purpose of this study was to determine and evaluate the microbial contamination of seafood products collected from retail markets at the Constantine region.

2 Materials and Methods

Sardines and shrimps were randomly collected from 10 fishmongers at different places in Constantine, Algeria. The collected samples were kept in sterile bags and brought in ice box to avoid the multiplication of microorganism. The samples include 32 sardines and 8 shrimps.

In all the samples, total aerobic bacteria counted, total and fecal coliforms, *Staphylococcus aureus*, *Salmonella*, sulphite reducing anaerobes bacteria, *Vibrio spp.* were determined. Under aseptic conditions, we weighed 25 g of each sample and homogenized it in a stomacher blender for 2 min at 150 revolutions/min with 225 ml of 0.1% peptone water (pH 7.0 ± 0, 2).

Detection methods for different organisms were based on the following international standards:

- Mesophilic aerobic counts (EN ISO 4833:2003).
- Lactose positive *Enterobacteria* and *Escherichia coli* counts (UNE 55683).
- Horizontal methods for the enumeration of coagulase positive *Staphylococcus aureus* counts (ISO 6888-1-1999).
- Horizontal method for the detection of *Salmonella* species (EN ISO 6579:2007).
- Determination of *Clostridia* (EN ISO 7937:2005).
- Horizontal method for the detection of potentially enteropathogenic *Vibrio spp*. (ISO/TS 21872-1, 2007).

The isolates were characterized for phenotypic and biochemical propreties for identification.

A test of significance of observed differences in levels of retrieved bacteria and fishmongers/ fish and seafood species parameters was conducted using a one way analysis of variance (ANOVA). Statistical significance was set at 5% ($p < 0.05$).

3 Results and Discussion

The present study showed out of the 40 seafood products samples examined, total aerobic bacteria ranged between 10^3 and 10^8 cfu g^{-1}. As it's shown in Table 1, none of sardines and shrimps were contaminated with *S. aureus* and *Vibrio spp*. Total and fecal coliforms were isolated from 31 (99.2%) of sardines and 8 (100%) of shrimps. *Salmonella spp.* was detected in one sample of sardines (3%), *Clostridium spp.* was isolated in 3 sample of sardines (10%) and 2 samples of shrimps (25%).

Tables 2 and 3, showed the mean of microbial contamination of sardines and shrimps in each fishmongers.

Table 1 Prevalence (%) of microbial contamination in sardines and shrimps in Constantine.

Seafood	Number of samples	Total coliforms (%)	Fecal coliforms (%)	*Salmonella spp.* (%)	*Clostridium spp.* (%)	*Vibrio spp.* (%)	*Staphylococcus aureus* (%)
Sardines	32	31 (99.2%)	31 (99. 2%)	1(3%)	3 (10%)	0	0
Shrimps	8	8 (100%)	8 (100%)	0	2 (25%)	0	0

Table 2 Microbial contamination of sardines in 10 fishmongers in the Constantine region.

	Number of samples	MAC mean (cfu g^{1})	P	TC mean (cfu g^{1})	P	FC mean (cfu g^{1})	P	SLM mean (cfu g^{1})	Cl mean (cfu g^{1})	*Vibrio* mean (cfu g^{1})	SA mean (cfu g^{1})
A.S		**10^7**		-		**40**		**ABS**	**-**	**-**	**10^4**
Fishmongers											
F1	3	10^5		$8x10^3$		$4x10^3$		Abs	Abs	Abs	Abs
F2	2	$2x10^5$		10^5		$5x10^3$		Abs	10^2	Abs	Abs
F3	4	$4x10^5$		$5x10^4$		$5x10^4$		Abs	Abs	Abs	Abs
F4	6	$9x10^4$		$9x10^5$		$2x10^4$		Abs	10	Abs	Abs
F5	3	$3x10^7$		$7x10^4$		$3x10^6$		Abs	Abs	Abs	Abs
F6	4	$2x10^5$	**0.219**	$3x10^4$	**0.605**	10^4	**0.397**	Pre	10^2	Abs	Abs
F7	2	$5x10^5$		10^4		$4x10^4$		Abs	Abs	Abs	Abs
F8	3	10^5		10^4		$6x10^3$		Abs	Abs	Abs	Abs
F9	3	$3x10^4$		10^4		$2x10^4$		Abs	Abs	Abs	Abs
F10	2	$4x10^4$		$5x10^3$		10^4		Abs	Abs	Abs	Abs

Table 3 Microbial contamination of shrimps in 4 fishmongers in the Constantine.

	Number of samples	TAMF mean (cfu g^{1})	TC mean (cfu g^{1})	FC mean (cfu g^{1})	SLM mean (cfu g^{1})	Cl mean (cfu g^{1})	Vibrio mean (cfu g^{1})	SA mean (cfu g^{1})
A.S		$5x10^7$	10^4	10^2	0	-	-	10^4
Fishmongers								
F1	2	$3x10^5$	$4x10^3$	$8x10^3$	Abs	Abs	Abs	Abs
F2	2	$6x10^5$	10^4	$5x10^2$	Abs	Abs	Abs	Abs
F3	1	10^8	10^5	$3x10^2$	Abs	$2x10^2$	Abs	Abs
F6	2	$5x10^6$	$3x10^4$	$5x10^3$	Abs	10	Abs	Abs

A.S: Algerian Standards (Official Journal of The Republic of Algeria, No35, 1998).
F: Fishmongers, MAC: Mesophilic Aerobic Counts, TC: Total Coliforms, FC: Fecal Coliforms, SLM: *Salmonella spp.*, Cl : *Clostridium spp.*, SA: *Staphylococcus aureus* , Abs: Absence , Pre: Presence.

In this present study, seafood products markets samples from 10 fishemongers showed higher number of bacteria. Statistical results showed that there is not a significant results between means of total aerobic bacteria, total and fecal coliforms founded in sardines from the 10 fishmongers (p> 0,05). The microbial contamination is high and similar in the 10 fishemongers.

The maximum microbiological limit for the total aerobic bacteria counts in fresh fish recommended by Algerian Standards (Official Journal of The Republic of Algeria, No35, 1998) is 10^7 cfu g^{-1}. Most of Sardines and shrimps markets samples were below this limit, but the mean of sardines samples (n=3) from the fishermongers number 5 and one sample (12.5%) of shrimps from fishermongers number 3 exceeded this limit (Table 2, 3).

The Higher microbial counts in some samples may be attributable to handling during harvest or processing.

The total bacteria count on fish rarely indicate the quality of the fish but it gives an indication of the risk of spoilage induced since each of these organisms had different ways of effecting health conditions of consumers of such contamination fish (Who 2007).

The maximum limit value for coliforms group bacteria in the fresh fish recommended by Algerian Standards (Official Journal of The Republic of Algeria, No35, 1998) is 40 cfu g^{-1} for sardines, 10^4 cfu g^{-1} (total coliforms) and 10^2 cfu g^{-1} (fecal coliforms) for shrimps (Table 1, 2, 3). The high rate found in the coliforms group bacteria showed that the fish analyzed were exposed to some kind of contamination. The contamination of fish from enteric bacteria of human and animal origin may also be responsible for various food spoilages (Empikpe, 2011). During handling and storage, or while transporting quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product, Adebayo-Tayo et al. (2012b). The quality of fish degrades; due to a complex process in which physical, chemical and microbiological form of deterioration are implicated (Adedji, 2012).

Shewan (1971) noted that coliform counts of recently caught fish were relatively low, but increased considerably during handling. Limit values of indicator bacteria for fecal contamination varies among countries. However, according to shewan (1970), fecal coliforms, an indicator of fecal contamination, should be less than 10^1 cfu g^{-1} (Fatma et al., 2006).

The presence of total and fecal coliforms indicated that the other harmful pathogenic microorganisms such as salmonella might be present in samples, Abu Henamuhammad (2008). It was found that one sample (3%) of sardine contained *Salmonella spp.* (table 1).Nearly similar results were obtained in Slovenia where shellfish samples were contaminated by one (0, 5%) strain of *Salmonella* (Biasizzo et al., 2005). In Nigeria *Salmonella* was isolated from two fish samples (10%) (Adebayo-Tayo et al., 2012a). In Egypt it was founded in 4 samples (10%) of shellfish, Ali and Hamza (2004) and in Bangladesh 4 samples (25%) were contaminated by *Salmonella*, Abu Henamuhammad (2008). Lower results were founded in seafood products (0% of *Salmonella*) in Poland, (Remigiusz et al.2012). Much higher results were obtained, in 100 fish and shellfish samples from the market and fish-landing conte in India, *Salmonella* was detected in 70% of fish, 59% of shrimp and 30% of oyster samples (Sallam, 2007).

The potential source of *Salmonella* contamination in seafood farms is likely due to poor water quality, farm runoff and fecal contamination from wild animals or livestock. In addition to poor distribution, retail marketing, handling, and preparation practices, high stocking, densities and high water temperature may be responsible for increased Salmonella contamination of shrimp (Wogu et al., 2010).

The presence of anaerobe bacteria presumed *Clostridium spp.*, may be related to the feacal contamination of marine environments where spores of the microorganisms permit their persistence (Remigiusz et al., 2012). Clostridia are ubiquitous in aquatic environments and they were isolated from water, sediments, fish and shellfish. These bacteria have been proposed as an alternative indicator of faecal contamination of marine environments and molluscs but the idea has not found an universal acceptance due to their high prevalence in marine water (Pasquale et al., 2012).

4 Conclusion

This study showed that seafood products from markets are generally unsafe for the consumers; the presence of contaminating bacteria could be attributed to cross contamination from environment, source and handling by the sellers. This finding may be considered as additional knowledge to enhance proper controlling of the storage life of fish, and fish products quality in Constantine. Safety of this kind of seafood can be guaranteed mainly by preventive measures and application of appropriates procedures of hygiene. Surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health.

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