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Assessment of Food Poisoning Bacteria in Some Frozen Fish and Fish Products

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Abstract

Consumption of frozen fish and fish products has increased worldwide. Levels of *Escherichia coli, Staphylococcus aureus, Salmonella* Typhimurium and *Listeria monocytogenes* in frozen fish products marketed in Cairo and Sharkia Governorates, Egypt, were investigated. A total of 150 samples including peeled shrimp, surimi, fish fillet, oyster and lobster tail (30, each) were examined. The overall contamination rate of frozen fish products was 6%. *E. coli*, *L. monocytogenes* and *S.* Typhimurium were identified in 2.7%, 2% and 1.3% of the examined samples, respectively. Health and food safety organizations should follow up fish products in all markets and apply the laws that prevent selling any fish products of unknown sources. Moreover, to ensure safety of fishery products, improvement of hygienic processing and handling from fish farming to markets is recommended.

Keywords: Frozen fish, *E. coli, S. aureus, S.* Typhimurium, *L. monocytogenes*

Introduction

In food industry, safety and hygienic quality are the most important issues related to human health. Food poisoning caused by seafood is the most frequently reported causing diseases ranging in severity from mild to chronic or even may lead to death [1]. Many foodborne outbreaks were reported worldwide, and in industrialized nations nearly 30% of the population suffers from foodborne illness yearly [2]. In the United States, 76 million cases were detected each year, of which, 325,000 cases received treatment at hospitals and about 5,000 deaths were reported [3]. In Europe, 5609 foodborne outbreaks were reported in 2007, of which, 3291 cases received treatment at hospitals and about 19 deaths occurred [4]. Food poisoning occurs by consumption of a poor quality foodstuff which is contaminated by one of the foodborne pathogens [2].

Outbreaks of foodborne diseases were related to many kinds of fish and its products. In New York from 1980 to 1994, 339 outbreaks related to fish consumption were reported and lead to 3959 cases of illness, 76 cases received treatment at hospitals and only four cases died [5]. From 1990 to 2002, 2472 outbreaks were reported by Center for Science in the Public Interest (CSPI), most of them were caused by seafood consumption (539 outbreaks including 6781 cases of illness) [6]. It is necessary to ensure hygiene and food safety for the consumer´s health and industry, however, *Salmonella* species, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes* (*L. monocytogenes*) and *Vibrio* species have been detected repeatedly in many fish products.

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E. coli O157:H7 is one of the most notorious foodborne pathogens, with an infectious dose of a few hundred cells [7]. *S. aureus* is a common pathogen that causes food poisoning outbreaks of great economic importance throughout the world [8]. It has been found widely distributed in nature, animals and humans. Moreover, *Salmonella* is one of the leading causes of bacterial foodborne illness in humans, causing over one million illnesses in the United States per year [9].

Since 1929, *L. monocytogenes* has been detected as an important opportunistic pathogens affecting humans and as a pathogen transmitted through food consumption since 1981 [10]. Its public health significance is attributed to the increased distribution in nature which is manifested by its wide host range. Classic procedures of culturing techniques of microbes including the biochemical characters and immunological techniques for the detection of pathogen-specific antigens are the basis of microbial identification [11]. The disadvantages of these classic methods are the time as it takes several days to be completed [12]. In addition, these methods are characterized by low specificity in selecting and identifying foodborne pathogens [13].

Great efforts were done to develop more rapid, cheap and sensitive methods for the isolation and identification of foodborne pathogens [14,15]. This study was carried out to estimate the presence of *E. coli, S. aureus, S.* Typhimurium and *L. monocytogenes* in some frozen fish products purchased from markets in Cairo and Sharkia Governorates, Egypt, to evaluate its microbial quality.

Material and Methods

Collection of samples

A total of 150 frozen fish samples including peeled shrimp, surimi, fillet, oyster and lobster tail were collected from markets in Cairo and Sharkia Governorates, Egypt. The collected samples were packed, identified, transferred in ice-box and immediately processed at the Laboratory of Animal Health Research Institute, Giza, Egypt.

Preparation of the samples

Twenty five grams from each sample were aseptically cut into small pieces and added to 225 ml buffered peptone water 0.1% [16]. The samples were then homogenized for 2 minutes. Ten fold serial dilutions were prepared for further analysis.

Bacteriological Examination

E. coli isolation

One ml of the sample homogenate from each dilution was inoculated into five tubes of 9 ml Lauryl Sulphate Tryptose broth (LST). The LST tubes were incubated for 48 hrs at 35°C then the tubes were examined for gas production. From each positive tube, a loopful of suspension was transmitted to Brilliant green lactose bile broth. The tubes were then incubated for 48 hrs at 35°C and tested for gas production for Colifom detection. A loopful from LST+ve tubes was transferred to *E. coli* (EC) broth tube, then it was incubated at 45.5°C for 48 hrs and tested for gas production [16]. A loopful from +ve EC broth was spread onto Levine's eosin-methylene blue agar plate (L-EMB) and then incubated at 35°C for 18-24 hrs. Plates were examined for suspected colonies of *E. coli* (violet flat colonies with dark center with or without metallic sheen). Suspected colonies were then subjected to biochemical identification using indole, methyle red, voges proskaur and citrate utilization tests [17].

S. aureus isolation

One ml from the sample suspension was aseptically transmitted to three plates of Baird-Parker agar, distributing it as following (e.g., 0.4 ml, 0.3 ml and 0.3 ml). The plates were then incubated at 35°C for 48 hrs [18]. Biochemical identification of suspected *S. aureus* isolates was performed using catalase activity test and coagulase test [19].

S. Typhimurium isolation

Incubation of the homogenized sample at 37°C for 18 hrs was carried out and then 0.1 ml of pre-enriched samples were transferred to 10 ml Rappaport Vassiliadis broth (RV) and incubated at 42.5°C for 24 hrs. Loopful from

enriched RV broth was separately streaked onto Xylose Lysine Desoxycholate (XLD) agar and incubated at 37°C for 24 hrs. Typical colonies were selected and streaked onto Triple sugar iron agar (TSI) and incubated at 37°C for 24 hrs [20]. Suspected colonies were subjected to biochemical identification using indole, methyle red, voges proskaur, citrate utilization and hydrogen sulphide production tests [21].

Listeria monocytogenes isolation

Twenty five grams of each sample were aseptically weighted and transferred to 225 ml of *Listeria* Enrichment Broth, University of Vermont Medium ($LEBUVM_I$) provided from biolife and then incubated at 30°C for 24 hrs. An amount of 0.1 ml from $LEBUVM_I$ was transferred to 10 ml of $LEBUVM_{II}$ and then incubated for 24 hrs at 30°C. A loopful from $LEBUVM_I$ and $LEBUVM_{II}$, each, were streaked onto plates of PALCAM agar and incubated for 48 hrs at 30°C [22,23]. Biochemical identification was performed using H_2S production, Catalase, Carbohydrate fermentation tests [24,25].

Results and Discussion

The results illustrated in Table (1) show the occurrence of some foodborne pathogens in peeled shrimp (n = 30). It was observed that the isolation rates of *S.* Typhimurium and *L. monocytogens* were 6.7% and 3.3%, respectively. None of the samples were positive for *E. coli* or *S. aureus*. These results are in agreement with those obtained previously by Ayulo *et al.* [26] who identified *S. aureus* in 20% of the examined samples.

Table 1: Prevalence of Some food borne pathogens in some frozen fish and fish products collected from Cairo and Sharkia markets

Type of samples	Number examined	*E. coli*	*S. aureus*	*S.* Typhimurium	*L. monocytogens*
Peeled shrimp	30	0	0	2 (6. 7%)	1 (3.3%)
Surimi	30	0	0	0	0
Fillets	30	3 (10%)	0	0	1 (3.3%)
Oyster	30	1 (3.3%)	0	0	1 (3.3%)
lobster tail	30	0	0	0	0
Total	150	4 (2.7%)	0	2 (1.3%)	3 (2%)

Regarding surimi, all the examined samples were free from the investigated pathogens (Table 1). These results are nearly similar to those obtained by Ayulo *et al.* [26] and Amagliani *et al.* [27]. Additionally, the isolation rate of *E. coli* was 10 %, while, *L. monocytogens* was isolated from 3.3 % in fish fillet samples. These results are nearly similar to previously reported studies [26,27]. In oyster, it was observed that the occurrence of *E. coli* and *L. monocytogens* was 3.3%, each (Table 1). These results are nearly similar to those recorded by Ayulo *et al.* [26] and Amagliani *et al.* [27]. All lobster tail samples were negative for the investigated pathogens. These results are relatively agreed to those obtained by Ayulo *et al.* [26] and Amagliani *et al.* [27]. According to the Egyptian Standards 516/2005 for shrimps, 889/2005 for fish fillet and 2800/2005 for oyster, all positive samples were rejected from retail for human consumption.

There is a correlation between *L. monocytogenes* in aquatic environments and human activity as the greatest infections of *L. monocytogenes* occur due to its ability to survive in the environment. Therefore, human listeriosis might be caused by fish consumption resulting in public health hazards. Raw products that are not subjected to heat treatment before consumption are considered a risk factor for *L. monocytogenes* infection [28]. Fish and its products could be contaminated by *L. monocytogenes* during raw meat and meat products processing. The results of the current study revealed the identification of *L. monocytogens* in 2% of the examined samples. Such percentage is lower than 6.1% obtained by Yu *et al.* [29], 5.3 %, 4.2 %, 5.6 %, respectively, for sauce pickled ,

cured and smoked products [30], 4.5 % [31], 7.8% [32] and 8.6% [33]. The obtained results for *L. monocytogens* are higher than1.1% reported by Uyttendaele *et al.* [34], while, Davies *et al.* [35] failed to detect *L. monocytogens.*

S. Typhimurium was isolated from different kinds of seafood, including fish and other fish products. The type of seafood affects the rate of occurrence of *S.* Typhimurium in fish products. Such assumption is supported by the highest prevalence reported in shrimp, mollusks and clams. Also, filter-feeding organisms are characterized by high prevalence of *S.* Typhimurium because a large amount of water was filtrated during their life cycle and the pathogen was accumulated in their tissues [36].

S. Typhimurium infection is also affected by human activity and the surrounding environment, therefore, contamination might occur at contaminated coastal areas and by food handlers [37].

The current results of *S.* Typhimurium were greatly lower than 31.7% obtained by David *et al.* [38], 15.5% which varies according to the type of fish products into 23.1%, 18.6%, 13% and 12.2% for oyster, fresh water fish, shrimp and marine water fish, respectively [39], 8 % for *Tilapia nilotica* and 16 % for *Mugil cephalus* [40]. Moreover, the obtained results for *S.* Typhimurium were in agreement to some extent with 2.02% isolation rate obtained by Yu *et al.* [29].

S. aureus is an opportunistic organism present naturally in the surrounding environment, contaminating food by cross contamination from utensils or persons even after cooking [41].Their presence in food indicates poor personal hygiene and poor manufacturing practices of the vendor [42]. In the current study, *S. aureus* was not isolated from any of the examined fish products. In contrary, Helmy *et al.* [43] reported *S. aureus* isolation rate of 14.5 % in ready to eat sea food, while Yu *et al.* [29] reported 8.1% occurrence of *S. aureus* in commercial cold food dishes.

Coliform bacteria, especially fecal coliforms (*E. coli*) are enteric bacteria, which present naturally in human intestine**.** It is considered as an indicator of food contamination by fecal contaminants from dirty equipment, handlers and contaminated water [44].The obtained results of *E. coli* were lower than that obtained by El-Sherief *et al.* [40] (12 %, for *Tilapia nilotica*; 4 % for *Mugil cephalus*). In contrast, Yu *et al.* [29] failed to detect *E. coli* in the examined fish samples.

Conclusion

Isolation of the investigated foodborne pathogens from frozen fish and fish products revealed the public health hazard potential and the examined samples did not meet the Egyptian Standards. Further studies are needed to evaluate the microbial quality of retail frozen fish. Continuous enforcement of hygienic conditions in areas of food handling, food contact surfaces, and personal hygienic practices should be followed to reduce the potential contamination of frozen fish and fish products.

Conflict of interest

None of the authors have any conflict of interest to declare

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الملخص العربي

تقييم بكتيريا التسمم الغذائي فى بعض الأسماك المجمدة ومنتجاتها

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فى هذه الدراسة تم فحص عدد ١٥٠ عينة من الأسماك ومنتجاتها المجمدة فى أسواق محافظتي القاهرة والشرقية وكانت العينات موزعة بالتساوي ٣٠ عينة لكل من (الجمبري المقشر – السوريمى – فيليه الأسماك – المحار – ذيول الاستاكوزا). وقد أظهرت النتائج وجود عدد ٤ عينات ملوثة بالميكروب القولوني النموذجي بنسبة تواجد (٢.٦٧ %), بينما تواجد عدد ٣ عينة ملوثة بميكروب الليستريا مونوسيتوجينز بنسبة تواجد بلغت (٢%) , في حين أن عينتين فقط كانتا ايجابيتين لميكروب السالمونيلا بنسبة تواجد (١.٣٣%). ولم يتم عزل الميكروب الذهبي العنقودي من جميع العينات. لذلك كان اجمالى العينات الايجابية لجميع ميكروبات التسمم الغذائي تحت الدراسة ٩ عينات بنسبة (٦%) من العينات المجمعة من الأسواق. لذلك فان السلطات الصحية يجب أن تهتم بصلاحية منتجات الأسماك في الأسواق مع منع تداول المنتجات مجهولة المصدر. إضافة الي الاهتمام بالأساليب الصحية في تداول المنتجات السمكية من عملية الصيد وحتى التصنيع والتداول في الأسواق.