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ScienceDirect

Procedia in Vaccinology 9 (2015) 44 – 49

Procedia in Vaccinology

www.elsevier.com/locate/procedia

8th Vaccine & ISV Congress, Philadelphia, USA, 2015

Study of the prevalence of *Staphylococcus aureus* in marine and farmed shrimps in Iran aiming the future development of a prophylactic vaccine

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Abstract. *Staphylococcus aureus* is the most important pathogen found in sea foods. Food poisoning in human may happen due to the consumption of aqua products contaminated with this bacteria and its enterotoxin. The procedures carried out to maintain and preserve the quality of these products, from the time they are fished and transported to stores until they are consumed, can play a major role in the generation and growth of pathogenic bacteria and toxins. A total of 300 samples were collected, including (fresh and frozen, farm and marine). Consistent with the Iran National Standards, a number of phenotypical and molecular assays were utilized for screening *S. aureus* in order to detect *Staphylococcus aureus.* They study was conducted from September 2013 to March 2014. Baird Parker agar containing egg-yolk and tellurite emulsion were used for isolation. Isolates were identified using the following criteria: production of coagulase, DNase, catalase, mannitol fermentation, hemolytic zone on 5% sheep blood agar, VP test and Gramstaining A total of 74 samples (24.6%), were contaminated with *Staphylococcus aureus*. Due to the presence of *Staphylococcus aureus* in shrimps, it is necessary to enforce quality control standards by the fisheries and carefully monitor fishing, farming, preparation, freezing, and transporting marine products, and ensure the health of workers. The results of this study also showed it is necessary to produce and develop a vaccine to prevent the disease and sea-food poisoning caused by *Staphylococcus aureus*.

Selection and peer-review under responsibility of the 8th Vaccine Conference Organizing Committee.

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doi:10.1016/j.provac.2015.05.008

Keywords: Staphylococcus aureus ; sea food ; shrimp; vaccine

1.Introduction:

Sea food is one of the most essential nutritional needs of every community. Often, consumption of contaminated shrimp cause gastrointestinal diseases in human (*1,2*). Staphylococcal food poisoning causes vomiting, diarrhoea,

and abdominal cramps within two to six hours after the ingestion of food contaminated with SEs *(3,4).* This bacterium does not require any particular nutritional or environmental factors for growth. The bacteria can also grow in substrates with a low water activity of 0.86, over a wide temperature range of 7 to 48 C, and at pH values ranging from 4.2 to 9.3 *(2).*

Several studies in other countries have investigated the potential paths of transmission of this dangerous strain by human carriers or environment, such as transport and packaging, contaminated hands of workers, and the contact of infected respiratory secretions with seafood products (*5,6,7,8*). In some parts of the world, more than 50% of food poisoning is caused by SEA. In Great Britain and America, SEA and SEB are the cause of more than 69% of all food poisonings *(5,6*). The symptoms are more severe in children, pregnant women, elderly, and patients who are undergoing tumor therapy or are taking immune suppressing drugs; due to quick digestion and proper absorption of protein and minerals, these population groups consume more shrimp, therefore the safety of these products becomes more critical (*7*). On the other hand, because of its tissue, shrimp meat has a high potential for corruption. Improper conditions during fishing and storage and non-standard transportation provide a good ground for pathogens growth (*7,8,9*). Unfortunately improper cooking method used for these products is the most important reason for causing disease*(5*).

All these factors increase the risk of gastroenteritis and food poisoning caused by contaminated food. Food borne diseases are a large group of the global diseases and are one of the most important problems in every community *(9*). *S. aureus* is considered as the third most important cause of food borne illnesses reported worldwide (9,10,11). This bacterium is one of the most common agents in food poisoning outbreak (*3,4*). In Iran, food poisoning outbreaks by *S.aureus* have increased during recent years. This might be due to changes in the environment, the development of the food service industry, and communal feeding. PCR-based techniques are commonly used for typing, as they are easy, fast, and cost-effective (1). There is no data published on the characterization of *S.aureus* strains in sea foods in Iran.

The purpose of the present study was to assess *Staphylococcus aureus* in shrimps supplied in Tehran fishery center.

2.Materials and methods:

2.1 *Samples Collection and Culture*

The experiments were approved by the Institute of Standards and Industrial Research of Iran (*12,13,14*). A total of 300 samples (fresh and frozen), including 150 marine shrimps, 150 farmed shrimps, with the healthy appearance were selected and studied from September 2013 to March 2014. The shrimp samples were caught from south seas (Persian Gulf, Oman Sea, Indian ocean) and aquacultures, and the shrimps were brought to the Tehran fishery.

In a sterile condition and near a flame, 1gr of each sample was cut with a sterile scalpel, was mixed with 9 CC Gulity (*s, aureuse* enrichment, Selective Media/ Merck, Germany) containing 0.1% potassium tellurite and the solution was suspended. Tubes were incubated at 37˚c for 24-48 hours. After passing the desired time, tubes that had deposits or were black, were cultured in Baird Parker (Merck,Germany) With 0.1% potassium tellurite and egg emulsion, though Linear Culture method; afterward, they were incubated at 37˚c for 24- 48 hours in sterile conditions.

To detect *Staphylococcus aureus* we studied a small black and shiny colony with oil zone, indicating *Staphylococcus aureus*. A slide was prepared from an isolated colony and Gram staining method was applied. For final verification of *Staphylococcus aureus*, citrated plasma was used to investigate the coagulase. *S ,aureus* catalase test was positive, ability to ferment mannitol tests ,DNase was posetive, hemolytic zone on 5% sheep blood agar and VP test were also performed.

For all the tests, *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were used as negative control and positive control, respectively (12).

2.2 *Statistical analysis*:

The prevalence of *S. aureus* isolates between eight categories was compared by using the Chi-square test (SpSS . 19). Differences between the prevalence rates were considered significant when $p<0.05$.

3. Results:.

Making use of biochemical assays, 74 *S. aureus* were isolated and grouped into four categories based on sea food sample origins, which were aqua products (fresh marine shrimp ,frozen marine shrimp, fresh farm shrimp, frozen farm shrimp; n ~300) As shown in Table1, a total of 74 (24.6%) out of 300 shrimp samples were found to be contaminated with *staphylococcus aureus*. *According to the analysis*, 22 % fresh marine shrimp, 37% frozen marine shrimp, 16% fresh farm shrimp, and 25% frozen farm shrimp were contaminated with *S. aureus* .

.Distribution of shrimp samples is shown in Table 1.

1-The presence of *Staphylococcus aureus* in the studied samples of shrimp

Studied samples	**Number of samples**	**Number of samples contaminated**
Fresh marine shrimp	75	16*(22%)
Frozen marine shrimp	75	28(37%)
Fresh farm shrimp	75	12 (16%)
Frozen farm shrimp	75	18 (25%)
Total	300	74(24.6%)

* The number in parentheses indicates the percentage

4. Discussion:

Food safety plays an important role in community health. Despite continuing advances in knowledge and techniques in food safety, consumption of contaminated food is still one of the main causes of many diseases. shrimp meat has excellent nutritional value, being rich in proteins, vitamins, and unsaturated fatty acids(15,16*).*

It is also extremely perishable and the safe consumption requires adequate sanitary conditions from the moment of catch, through preparation, sale and consumption (*7,17,18*). In the countries which keep adequate records of diseases transmitted through food, eating contaminated shrimpis responsible for a significant number of disease outbreaks (*19*,20,21). Presence of pathogens is difficult to detect and therefore good hygiene is very important. The quality and

safety of shrimp can be directly influenced by the lack of hygienic habits of fish handlers and contact with contaminated work surfaces, including benches, tables and unwashed knives (*7,22*). Various factors pose a condition

of risk to sea food safety and they range from contamination from the environment where it is caught up to contamination by the consumer before eating (*20*,23).

According to NG *et al*, potentially pathogenic bacteria present in foods can reach high numbers without necessarily producing noticeable alterations in features, odour, or taste(24).

Based on our studies *S.aureus* was present in 74 (24.6%) out of 300 samples. Frozen marine shrimps were found to be more contaminated than other shrimps 28(37%).

The most frequently contaminated sea foods were frozen marineshrimp. It might be due to the source of contamination, including the pollution present in marine product transportation systems and freezing systems from main source to the destination (Tehran fishery). Long distance, temperature conditions, improper storage during transportation, inappropriate accumulation and packaging of frozen shrimps are the other reasons for the high levels of contamination of frozen shrimps compared with fresh shrimps. *Staphylococcus aureus* transmission from contaminated surfaces to seafood products can also contaminated them as well (*15*. In addition, *S. aureus* was detected in aqua products at levels as high as those in staphylococcal food poisoning cases by raw fishery products reported in many countries *(7,8,10,19).* Microbial growth is affected by environmental factors, such as pH, temperature, and water activity.

In Japan, the foods which are most frequently involved in staphylococcal food poisoning are sushi (raw fish) and lunch-box meals that contain multi-ingredient foods *(16,17).* In Italy, *S. aureus* was mainly detected in meat products *(2).* The major foods involved in outbreaks in Korea from 1996 to 2000 were meat, raw and undercooked sea food, multi-ingredient foods, and lunch-box meals *(11).* Monitoring RTE (ready to eat) food contamination by *S. aureus in* Korea, it was found that 19.8% of the raw fish was contaminated with *S. aureus.(20,21)*. Obviously, contamination of ready to eat foods in fishery products has been less than ever.

In a similar study carried out on aqua products in India, the rate of contamination with *S. aureus* was 17% which is much lower than our finding (22). A study on marine products in Brazil reported high rate of contamination with *S. aureus* (7*,18*). Many of other researchers reported lower rates of *S. aureus* than our results(16*,20,21,23).*

In conclusion, our study examined the trends of sea food contamination by *S. aureus*. The results obtained in this study can help us understand the distribution and prevalence of *S. aureus* among aqua products and can be used as a useful database for epidemiological purposes. Our further studies will examine the antimicrobial resistance, and assess the relationship between toxin type and the antimicrobial resistance of the isolates that were obtained in this study. Food monitoring needs urgent attention, although more study is needed to verify these results .It is necessary to produce and develop a vaccine to prevent diseases and sea-food poisoning caused by *Staphylococcus aureus*. Among the many infections to which the elderly ,children, and patients who are undergoing chemotherapy or are taking immune suppressing drugs therefore, some can be prevented by administration of appropriate vaccines. Vaccination of these groups of people is one of the most effective ways of preventing sea food borne disease.

Acknowledgements:

This work was supported by Tehran university of medical science and Management Program (240.3331) in public Health Faculty. We appreciate Fisheries organizations in Iran for providing samples.

Author contribution statement

Arfatahery .N.(* ***Corresponding Author's*** *)and*
Mirshafiey .A . professor advisor,
Arfatahery .N and Zeinolabedinizamani *.M. wrote the main manuscript text and* Abedimohtasab .T, *prepared Table 1 and* Cooperation in the microbiological experiments*. All authors reviewed the manuscript."*
This article is part of a Thesis N. Arfatahery.
The authors declare that there is no conflict of interests regarding the publication of this Manuscript.

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