RESEARCH ARTICLE **(Open Access)**

Evaluation of the presence of *Staphylococcus aureus* coagulase-positive in food products

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Abstract

The presence of *Staphylococcus aureus* coagulase-positive as an important pathogen in food products is the cause of infection and intoxication from food. Samples submitted for this thesis were collected over a period of one year from food business operators in Kosovo. During this period, 178 samples were collected which were analyzed from a microbiological aspect for the presence and count of the pathogen *Staphylococcus aureus*. Samples were taken by inspectors of the Food and Veterinary Agency and analyzed at the Food and Veterinary Laboratory in the Department of Food Microbiology using the horizontal method of counting coagulase-positive Staphylococci (*Staphylococcus aureus*) - ISO 6888-1: 1999. The presence of coagulase-positive *Staphylococcus aureus* coagulase-positive in food products is an indicator of poor hygiene during treatment or processing of food products, and being recognized as strong toxin producing bacteria in food products, it can be a cause of foodborne illnesses. The final result shows that five (5) samples out of 178 or 2.8%, have a positive result. From samples of milk and milk products, three (3) were positive, while from samples of meat and meat products two (2) proved to be positive. Results of samples of other food products were all negative.

Keywords: Staphylococcus aureus; enterotoxin; foodborne intoxication

1. Introduction

The dose required to produce an infection varies with the type of microorganism, even though the microorganism will usually multiply in the GI tract or some other organ of the body to produce the infectious disease [2].

Many bacteria, such as S. aureus, produce proteins (exotoxins) which modify, through enzymatic action, or in other words destroy certain cell structures. The effects of exotoxins usually are acute, since they are potent enough to result in serious consequences (i.e. death) [7].

Staphylococcal food poisoning is one of the most common types of foodborne disease worldwide. It is intoxication, resulting from the ingestion of food containing one or more preformed staphylococcal enterotoxins [2].

Staphylococci are Gram-positive cocci, 0.5-1.5 µm in diameter, and can occur as single cells, in pairs, or as clusters. *Staphycoccus* can be differentiated from other genera in the family on the basis of the giuanine plus cytosine (30-39 mol% G + C) content of the DNA, the presence of glycine in its peptidoglycan and teichoic acid in its cell wall, and ability to grow anaerobically [2].

Humans are the principal reservoir of *S. aureus*. About 30-50% of healthy individuals carry *S.aureus* in their nasal passages, throats, hair, and skin, 40-50% of the isolates being enterotoxin producers [2].

A common source of contamination of dairy products is from cows' udders, particularly from animals with staphylococcal mastitis. Essentially all raw foods, especially raw meats and poultry, can be contaminated with staphylococci. They may persists on raw meats but grow very poorly, as they are poor competitors. In foods that provide satisfactory medium, staphylococci can grow to sufficient numbers to produce enterotoxin if the foods are not refrigerated. These organisms can be transferred to

equipment; if the equipment is not adequately cleansed before use, the organisms can be transferred to foods. Heating is the most effective way to inactivate *S. aureus* in food. Heating meat to an internal temperature 73.9-76.6°C should be sufficient to inactivate any staphylococci present. The time-temperature treatments used to pasteurize milk are adequate to destroy the organisms. The *D*-values (time required to destroy 90% of organisms) of *S. aureus* in skim milk at 60°C and 65.5°C are 3.44 and 0.28 minutes, respectively.

S. aureus is relatively resistant to drying. Nonfat dry milk and foods containing nonfat dry milk have been implicated in staphylococcal food-poisoning outbreaks. Staphylococci present in the milk may survive spray drying, depending on the temperature, the moisture content of the product, and the strain of S. *aureus* [2].

The agents responsible for staphylococcal food poisoning are the staphylococcal enterotoxins. Nine serologically distinct enterotoxins have been identified based on their reactions with specific antibodies. They are designated enterotoxins A (SEA), B (SEB), C (SEC), D (SED), E (SEE), G (SEG), H (SEH), I (SEI), and J (SEJ). Minor antigenic variants of SEC have been described and they are SEC_1, SEC_2 and SEC_3. There is no enterotoxin F (SEF) because toxic shock syndrome toxin (a major causative agent of toxic shock syndrome) was misidentified as SEF when it was first isolated. SEA is the enterotoxin most frequently associated with staphylococcal foodborne outbreaks, with SED being the second most frequent. Analysis of outbreaks indicated that unidentified enterotoxins exist [2].

The enterotoxins are quite heat resistant. The degree of heat resistance depends on many factors, including the type of enterotoxin, purity of the preparation, amount of toxin, pH, and menstruum. Crude toxins preparations are more heat stable than purified toxins, while hat inactivation is faster in buffer than in culture media and foods. However, thermal process treatments employed by the canning industry are adequate to destroy the quantity of enterotoxins usually involved in food-poisoning outbreaks. Staphylococcal food poisoning resulting from canned foods was due to inadequate processing or recontamination of improperly sealed cans after retorting. The enterotoxins are not inactivated during pasteurization. Very little loss of SEA and SED in milk or cream occurred after pasteurization at 72°C for 15 seconds. Spray-drying processes used for milk are also insufficient to inactivate the enterotoxins. Spray-dried milk has been involved in several staphylococcal food-poisoning outbreaks. In general, the heat resistance of SEA is higher than that of SEB, which is higher than that of SEC.

It has been shown that gamma irradiation processes used for pasteurization or sterilization of foods may not be sufficient to inactivate the enterotoxins. More than 2.7 and 9.7 megarad (Mrad) was required to reduce the concentration of SEB in buffer and milk, respectively, by tenfold. In lean minced been slurries, 27-37% of SEA remained after a dose of 8 kGy. The more concentrated the beef slurry, the less the SEA was inactivated [2].

Growth and enterotoxin production by *S. aureus* are influenced by a variety of environmental and nutritional factors including temperature, pH, water activity (a_w), inoculum size, atmospheric composition, carbon and nitrogen sources, salt levels, and competing microflora. Generally, growth is necessary for enterotoxin production, although enterotoxin production does not always accompany growth, especially in foods. The amount of enterotoxin produced is dependent on staphylococcal strain and enterotoxin type [2].

2. Material and Methods

The working method is based on the microbiological analysis of some food products intended for human consumption. For sampling and test method ISO standards and guidelines of Codex Alimentarius were used. The work plan included 178 different samples which were analyzed in terms of the presence and bacteriological count of the pathogen *Staphylococcus aureus*.

Among the many factors that affect the propagation of pathogens we've got to factor basic hygienic handling of

food products surveyed. Mainly samples taken were from factories, processing food products.

2.1 Sampling

79 samples of raw milk and milk products were collected. The analyzed samples include raw and processed milk, cream, yoghurt, cream cheese, ice cream, etc.. Also 91 samples of meat and processed meat products were tested. Products include fresh cattle meat, beef or swine prosciutto, chicken legs and thighs, fresh beef patties, mechanically de-boned meat (MDM), poultry MDM, salami, pate, hotdogs, suxhuk (traditionally cured dried meat), etc.. Eight more samples which were analyzed included baby food and soups.

2.2 Sample transport

The samples were packaged in polyethylene bags and were transported using a hand-held refrigerator, in 4^0C temperature. Samples were accompanied by a supporting document which contains data on where the sample was take, the type of sample and who the sample was taken by.

2.4 Sample delivery

The sample is delivered together with the supporting document at the sample reception area of the Food and Veterinary Laboratory of the Food and Veterinary Agency where it is coded based on the Procedures of the Quality Control Management System according to ISO 17025:2005.

2.5 Horizontal method of counting Staphylococcus coagulase-positive (Staphylococcus aureus) – ISO 6888-1:1999

10 g/ml sample is taken and mixed into 90 ml peptone water, its put into the stomacher in a filtered stomacher bag and is diluted in a series. After the dilution it is transferred onto plates with selective media.

2.6 Inoculation

Using a sterile pipette, transfer 0.1 ml of the sample being tested if it is liquid, or 0.1 ml of the initial preparation in case of other products, in two agar plates. The procedure is repeated for dilution 10^{-2} if necessary. The inoculate is carefully and quickly spread onto the surface of a 90mm Baird-Parker agar plate, avoiding the sides of the plate. The plates rest for 15 minutes to dry at room temperature.

2.7 Incubation

The prepared plates are turned over and incubated at 35 ºC – 37 ºC for 24 ± 2 h. After incubation for 24 ± 2 h the plates are checked, if there is no growth they are reincubated at 35 ºC – 37 ºC for an additional 24 ± 2 h. After this period, every typical or atypical colony is counted [1]. Only plates containing a maximum of 300 colonies and which have 150 typical or atypical colonies in the previous dilutions. One of the plates must contain at least 15 colonies. A specific number of colonies is chosen for confirmation A (5 colonies of only typical colonies present and 5 colonies if only atypical colonies present and 5 typical and atypical of both kinds of colonies are present).

2.3 Confirmation

Chosen colonies are taken from the surface using a sterile loop and are transferred into a tube or bottle containing Brain-Heart Infusion. It is incubated at 35–37 ºC for 24 ± 2 h. 0.1 ml of each culture is aseptically added into 0.3 ml blood plasma (rabbit plasma coagulase test) in tubes and is incubated at 35–37 ºC. Tilting the tube, the plasma is examined for clotting after 4h-6h of incubation and if the test is negative it is re-examined after 24 hours.

The test is coagulase positive if the volume of clotting is more than half of the volume of the liquid. In each vial of plasma are added 0.1 ml of BHI and is incubated without inoculate, this test is negative and is used as a control [6].

3. Results and Discussion

The researched parameter *S. aureus* in food products such as: milk and milk products, meat and meat

products, as well as other products such as baby food etc., results as follow.

Staphylococcus aureus in meat and processed meat products is present in 2 of a total of 50 samples tested which is 4%. Products which were outside of limits were suxhuk (1) and salami (1). Of 41 samples tested of unprocessed meat 0 samples were outside of allowed limits. *S. aureus* grows very little in unprocessed meat because it is a poor competitor [2]. Some products such as MDM and chicken MDM had a presence of the microotganism, but it was below the allowed limit according the criteria used. The total number of milk and processed milk products was 74, of those 3 or 4% were outside of the limits. In this case we can conclude that either the thermal treatment factor did not have an effect in destroying the *Staphylococcus aureus*, or the product is contaminated during processing, packaging or storing.

In unprocessed milk and milk products samples, of 5 tested samples 0 were outside of limits. Other tested samples, baby food and soup, resulted negative for the presence of *S. aureus*. As far as the baby food is concerded, this result was expected since a special attention is given to it during processing. In the table below, you can see the results of the samples analyzed which had the highest load of *S. aureus*.

Table 1. Samples with highest load of *S. aureus*

No.	*Protocol no.*	*Sample type*	*Result*
1	239	Industrial Suxhuk	***6.46x10^4 cfu/gr***
2	265	Salami	***>300x102 cfu/gr***
3	216	Trapista cheese	***>300x102 cfu/gr***
4	257	White cheese	***>300x102 cfu/gr***
5	335	White cheese	***2.80x104 cfu/gr***

From the results we can conclude that cheese has the highest values of this parameter compared to other samples, as regards the processed food samples. Based on Microbiological Criteria of EU and Kosovo, three (3) samples result out of limits which are $10^2 – 10^3$ cfu/10 gr. The presence of this pathogen is concerning since toxins are a product of their activity. In this case we have lack of safety of raw materials, hygiene during processing and storage [8, 9].

Spoilage of food is considered any change that makes it unacceptable for human consumption [4]. Most food technologies used for food preservation are designed to destroy or inhibit spoilage microorganisms, in particular certain species of bacteria and fungi. From a food safety perspective, the use of preservation technology does not guarantee food is free from pathogenic or toxigenic microorganisms. Although many food preservation technologies also destroy or inhibit pathogenic or toxigenic microorganisms, this is not always the case. If not properly utilized, some food preservation technologies may actually increase the risk from pathogens or toxins. In other words, food preservation is not synonymous with food safety. A broader term known as *food protection* is often used to encompass both food safety and preservation. It is important for food safety professionals to understand the basic scientific principles that underlie technologies for food protection [7].

The intrinsic and extrinsic parameters that govern microbial growth form the basis of the hurdle concept. Hurdles are technological barriers to the growth and reproduction of microbial agents. A single-target approach to hurdles is using only one technological barrier to control microbial growth. The use of multiple technological barriers to control microbial growth is a multitargeted approach, particularly when each hurdle is aimed at a specific target of the microbial cell structure and/or [5].

Food science professionals must carefully design, test, and scientifically verify as effective the food protection technologies used in the hurdle approach. Because minimally processed foods imply the use of less severe preservation treatments, microbial agents are often inactivated – not destroyed, as happens with complete sterilization. Food protection engineers and technologists must characterize the degree of uncertainty and understand the limits of the multiple hurdles used. This is especially important for the control of foodborne pathogens and toxigenic microorganisms. Minimally processed foods must not allow for increased risk of foodborne illnesses [7].

When we speak about factors which inhibit the development of pathogenic and toxigenic microorganisms, this includes a large number of them such as the cleaning of the working equipment and their disinfection, removal and decontamination of waste generated during work, personal hygiene of staff that is directly involved with production, health of working staff, proper heat treatment of products, safety and cleaning of the raw material, avoiding cross-contamination etc.. The impact of these factors comes into play in food processing factories, meaning in environments with greater production capacity. But it can also occur in the production conditions in family environments. Based on methods of processing, we can say that industrial processors have better conditions to avoid contamination.
The epidemiology related to the pathogen researched in this case is very present in our country, data shows that most cases of infectious diseases are or a biological nature specifically from food.

4. Conclusions

The laboratory results showed that most samples taken had results within the allowed limits. This fact should not be enough for those who are responsible for the control of food products. Research should be continued to find the defects and eliminate even the small number of cases which resulted positive in order to fulfill the requirement for all products to be safe.

Of the samples tested the majority were within the level of the Microbiological Criteria, except three samples of processed milk. The composition or type of food product is important regarding to the conditions for growth of *S. aureus*. It is noticed that milk and milk products are more favorable to the growth of this pathogen whereas in meat and meat products this pathogen develops very slowly. From these findings we can say that milk and milk products must be paid special attention to in order to combat this pathogen.

Hygienic factors have contributed to the growth of pathogenic bacteria during processing, as well as other factors such as: pH, a_w, temperature, etc.

Laboratory results or abovementioned findings are an argument for the obligatory implementation of Good Work and Hygiene Practices as well as the HACCP system for all businesses which are in contact with food. Also, other factors such as packaging, storage, distribution are elements which increase the risk of contamination by pathogens.

From the findings, it has resulted that besides the controls according to national plans by the competent authority routine examination of operators is necessary in order to continually follow the eventual changes such as permanent record keeping of the presence of toxigenic pathogens in food products.

Milk producers must pay special attention to the reception of raw materials such as fresh milk which can contain a high load of pathogens. They must educated and inform the farmers as their clients to pay attention to the hygienic conditions during milking of the animals, storing and transporting to the processing plant. The processing must be done according to rules foreseen to guarantee product safety from *Staphylococcus aureus* and other pathogens.

Application of HACCP system by Food Business Operators presents a special importance in raising the hygiene level in the process of production and processing. This system represents the highest level of self-control of operators which produce or trade foods. Our country, in the future, should lay as a legal criterion the implementation of HACCP by producers, processors and traders of food. The establishment of this criterion increases the general level of safety of products for consumers in the whole country.

5. References

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