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Staphylococcal Enterotoxins

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Abstract Enterotoxins are macro-proteins produced by pathogenic bacteria that act on the gut of humans or animals. They are harmful for the consumers of milk and dairy products because they cause diarrhea with or without tissue damage. These molecules are antigenically and structurally different from each other. They can contaminate all types of foods (raw or cooked). This is the case of pastries, cheese, cooked dishes. The bacterium that produces these toxins is mainly present in milk and dairy products from mastitis cows. It is a disease born from the contamination of ruminant udders by golden *staphylococci* and other types of coagulase-producing *staphylococci*. Indeed, during the manufacturing process, *staphylococci* develop and thus produce enterotoxins. This article describes enterotoxin-producing organisms and environments that are conducive to their development, types of infections caused by staphylococcal enterotoxins, clinical signs and diagnostic.

Keywords *Staphylococcus aureus*, Enterotoxin, Enterotoxicosis, Food poisoning, Milk, Dairy products, Mastitis, Food, Contamination, Coagulase

1. Introduction

The bacterium that produces enterotoxin may be present in milk and dairy products from mastitis cows [2, 11, 15, 25, 32]. It is a disease born from the contamination of ruminants udders by *Staphylococcus aureus*; milk is then contaminated as well as products derived from this milk. Staphylococcal enterotoxins are toxins that are harmful to the consumers of milk and dairy products, hence the importance of detecting them in foods before they are placed on the market [24, 26]. Staphylococcal enterotoxins can contaminate any type of food (raw or cooked), such as Cheese and cooked dishes. The aim of this article is to summarize informations gathered from litterature on the enterotoxins, including the potential agents responsible for food poisoning.

2. Description of Enterotoxin Producing Staphylococci

Staphylococci are Gram-positive *cocci* usually classified in clusters. Currently, there are 44 species of *cocci* [2, 3, 23]. *Staphylococcus species*, more commonly known as *staphylococcus aureus*, is generally distinguished from other *staphylococci* called coagulase-negative *staphylococci* by the presence of coagulase [23]. *Staphylococcus. aureus* is a very important germ in community and nosocomial infections. They are ubiquitous bacteria on the skin, mucous membranes and nasopharyngeal sphere in warm-blooded animals (mammals and birds) and especially humans. These bacteria are also isolated from different environments such as soil, freshwater, sea, dust, kitchen, refrigerator, hospital and food preparation workshops. This germ is very important in infections both in humans and animals. *Staphylococcus. aureus* is one of the main pathogens involved in outbreaks of community food poisoning associated with the consumption of meats, sausages, milk and dairy products. Some strains of *staphylococci* are known to cause food poisoning around the world because they produce heat-stable enterotoxins [21, 28]. Study of Seo and Bohach [29] showed that 82.4% of staphylococcal strains derived from milk and 100% from food poisoning are enterotoxigenic.

2.1. Types of Staphylococcal Infections

Staphylococcus aureus may also be the cause of pyrogenic infections of the skin and mucous membranes in humans, septicemia, food poisoning and acute enterocolitis. The responsibility of *staphylococci* other than *S. aureus* in food-born outbreaks is difficult to assess because they are not usually sought after. Contamination with *S. intermedius* is unlikely because it is isolated only in certain animal species (dogs, cats, horses, pigeons, foxes and minks). *Staphylococcus hyicus* and coagulase *staphylococci* are the most common contaminants. They are isolated in cow's milk

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[6, 7]. *Staphylococcus hyicus* is also isolated from meats of pigs, cattle, poultry and horses. It can therefore contaminate foods and be problematic for identification because it shares some important traits with *S. aureus*. They appear to produce smaller amounts of enterotoxins than *S. aureus*, and these are sometimes non-identifiable by the usual methods. Finally, the rare strains of coagulase *staphylococc*i implicated in foodborne illness could be mutant strains of *S. aureus* that have lost their ability to produce coagulase (increasingly feared in hospitals because of antibiotics) or Staphylococci having reached immunocompromised subjects. They are:

- ✓ *S. epidermidis*, an opportunistic pathogen;
- ✓ *S. saprothyticus*, a predominant species in urinary tract infections in young women (cystitis);
- ✓ *S. warneri*, in some human infections (septicemia, endocarditis, conjunctivitis, urinary tract infections).

When staphylococcal infections occur, it is usually possible to demonstrate that the staphylococcal strain in the contaminated food is the same as the one present on the hands of those who handled it.

3. Favorable Environments for the Development of *Staphylococci*

Foods that facilitate the growth of *staphylococci* are mainly pastries, ice creams, processed foods such as hams, dough, rillette and potato, poultry and tuna salads. Cooked products contaminated after cooking (minced meat, fish, sliced cold cuts); products with reduced water content (cured meat, dried and smoked fish, milk powder), cheese, egg products, mayonnaise, dairy products (eg. condensed milk), creams and ice creams [13, 24]. Contaminated food has the same appearance (smell and taste) as healthy food. There has even been poisoning after eating canned vegetables and fruits.

4. Bacterial Enterotoxins

According to Lucas et al. [22], an enterotoxin is a toxic substance produced by an organism, especially certain bacteria, such as *cholera vibrio*, *Escherichia coli*, *salmonella*, *clostridium perfringens* and *shigella dysenteriae*. These toxins are likely to cause intestinal disorders during its dissemination in the digestive system. They adhere to the intestinal epithelium of the small intestine and prevent the absorption of *Na+* and *Cl-* ions promoting fluid leakage. Enterotoxins belong to a large family of pyrogenic toxins. Bacteria producing this toxins may be present in milk and dairy products from mastitis cows [25, 27]. This is a disease borne from the contamination of the ruminants udders by *staphylococcus aureus*; the milk is then contaminated as well as the products derived from that milk [19, 27]. Indeed, during the manufacturing process, *staphylococci* develop and produce enterotoxins. In the agri-food industry, professionals ensure compliance with regulatory criteria to ensure consumer safety. But these enterotoxins are resistant to most treatments used and remain present despite freezing or cooking. It is therefore very important to detect them. The foods in question are very varied. These are often cold cuts, eggs and dairy products. The contamination, almost always exogenous and of human origin, consists of a lack of hygiene that can be located at any point of the food chain. The main germs involved are two in number. *Staphylococcus aureus* is the first. Between 30 and 60% of *S. aureus* strains isolated from human infection, belonging to the Phages III and IV groups, produce enterotoxins and are responsible for boils and paronychia.

The pathogenicity of *Staphylococcus aureus* is also related to the production of toxins called enterotoxins. These, called staphylococcal enterotoxins A, B, C, D, E, etc.), are preformed in the food during bacterial multiplication and propagation. These different enterotoxins have been highlighted [1, 9]. To date, only 6 types (A-H) of enterotoxins have been identified. Most of staphylococcal enterotoxins are resistant to 100°C temperature for 30 minutes and to gastric acidity. These different serotypes are antigenically distinct. However, some authors such as [5, 20], have shown that there is an antigenic relationship between type A and E staphylococcal enterotoxins; type B and C enterotoxins [17]. These authors believe that the molecules of these enterotoxins contain fractions of antigens determining only the specificity of the groups. Thus, enterotoxins type A and E; B and C are antigenically related to each other [31].

Analyzing staphylococcal strains isolated from food poisoning cases producing enterotoxins, [12, 20] found that serotypes A and D predominate, followed by serotypes B. In addition, strains producing enterotoxin type C are often linked to dairy products that cause food poisoning [4, 8, 27]. On the other hand, in the study conducted by French researchers [18, 19] it was demonstrated that the predominance of enterotoxin type A in food poisoning situations.

5. Enterotoxicosis (Diseases Caused by Enterotoxins)

Of bacterial origin, in 60 to 80% of cases, acute infectious diarrhea is a communicable disease that almost always participates in a fecal-oral cycle. The epidemiological modalities vary from one continent to another depending on the development level of the health infrastructure. In countries with low levels of hygiene, bacterial diarrhea is responsible for more than 4 million deaths a year. Their transmission is favored by the absence of collective facilities necessary for the disposal of wastewater and the distribution of drinking water, but the exchange of enteric germs results mainly from direct human contact and frequent pollution of drinking water. In industrialized countries where sanitary facilities are satisfactory, direct transmission of enteric germs is rare and their dissemination by drinking water is

exceptional. On the other hand, we are witnessing an increase in food poisoning reflecting major sociological changes over the last quarter of a century. The emergence of collective catering and new methods of food production, processing and distribution has deeply changed individual and collective behavior regarding meal schedules and locations. These changes, accompanied by new demands on the quality of products, the hygiene of their environment and the safety of the consumers, are at the origin of the development of food bacteriology.

5.1. Clinical Signs

Incubation period is brief (one to four hours), symptoms appear suddenly, sometimes even before the end of the contaminating meal. Researchers perform a chart of acute gastroenteritis with nausea, vomiting, abdominal pain and diarrhea. Fever is absent, but the risk of dehydration is high. Hypotension or cardiovascular collapse may complicate the process. These rare accidents occur at the extreme ages of life. As a rule, symptoms improve in six to eight hours.

Staphylococcal enterotoxins increase the intracellular concentration of cyclic adenosine monophosphate. This results in a disruption of the cellular hydroelectrolytic exchanges at the origin of the diarrhea, but no cellular alteration or villous destruction is observed. Diarrhea results from ingestion of a preformed bacterial toxin in the food. The onset of symptoms is observed early after absorption of the food. There is a dose-response relationship, the damage is more severe and the incubation time shorter than the amount of toxins ingested would is significant.

The amount of enterotoxin swallowed determines the timing of symptoms and their severity. It takes 500,000 to 5, 000,000 germs /g to trigger the troubles. Symptoms appear quickly (1 to 2 hours, up to 6 hours); at the beginning there is abundant salivation, nausea, headache, vomiting, sweat, neurological disorders, severe abdominal pains and diarrhea. There is usually no elevated temperature, sometimes slight hyperthermia (up to 38℃) or hypothermia (severe cases in infants and old men). Severe cases are accompanied by dehydration and shedding of blood and mucus in the stool and vomiting.

The experiment carried out in humans of 70 kg showed that the symptoms appear from 350 ng of enterotoxins consumed. It is estimated, however, that in sensitive individuals, 100 ng may be sufficient to trigger clinical signs. Animals are less sensitive than humans to the action of enterotoxins.

5.2. Diagnostic

Until now, the detection of staphylococcal enterotoxins has been achieved by biological and immunochemical tests [9, 10, 14, 16, 27]. The immunochemical method of detection is done after extraction and then dialysis. These different methods have been problematic in communication and trade between countries in border food controls. To address the need for harmonization and international regulatory requirements, a panel of experts has been established to develop a validated staphylococcal enterotoxin detection method for five food categories. Thanks to the financial support of the European Commission, the first method using the standardized enzyme-linked immunoassay has been developed. These kits are marketed. This first standardized detection method is now at the heart of the draft European and International Voluntary Standard ISO 19020 defining performance criteria for diagnostic kits. With this European voluntary standard, laboratories will be able to analyze food products. The proposed method consists in extracting and then concentrating the enterotoxins in order to better identify them by the said method [32].

6. Conclusions

Staphylococci are pathogens that cause food poisoning around the world. Milk and dairy products are largely important vectors of these germs. Carried by cow with mastitis, which causes contamination of the milk and its products. The heat treatment, the respect of good hygiene practices and the respect of the cold chain are of course the major axes allowing the considerable reduction of staphylococcal germs in food, but have no effect on enterotoxins because they are thermostable. There are several methods of diagnosing enterotoxicosis which include biological, immunochemical and enzyme-linked immunoassay. Among these methods, only the biological test does not require the extraction of enterotoxins. On the other hand, the immunochemical and immuno-enzymatic tests require extraction and then dialysis.

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