

***Staphylococcus aureus*: a threat to food safety**

Staphylococcus aureus: uma ameaça à segurança alimentar

Staphylococcus aureus: una amenaza para la seguridad alimentaria

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Abstract

Foodborne Diseases (FBD) affect millions of people worldwide. They are caused mainly by bacteria of the genus *Staphylococcus*, mainly *Staphylococcus aureus*. Among these, some produce enterotoxins that are resistant to high temperatures and can contaminate various types of foods such as milk and dairy products, in addition to meat products and others. For these reasons, it is necessary to study *S aureus* and Staphylococcal Enterotoxins (SE), which are the main causes of FBD, and the development of actions and new products that maintain the health of food, especially milk, dairy derivatives and meat products, in order to reduce contamination. In addition to the study of more efficient techniques for detecting these contaminating agents. Among these techniques, molecular techniques stand out, which currently have been shown to be increasingly sensitive and specific. In general, the association of good hygiene practices and molecular detection techniques are fundamental for maintaining food health, avoiding losses in the industry and serious health problems.

Keywords: Foodborne diseases; Food poisoning; Toxins; Good handling practices.

Resumo

As Doenças Transmitidas por Alimentos (DTAs) afetam milhões de pessoas em todo mundo. Elas são causadas principalmente por bactérias do gênero *Staphylococcus*, principalmente *Staphylococcus aureus*. Dentre esses, alguns produzem enterotoxinas que são resistentes a altas temperaturas, podendo contaminar vários tipos de alimentos como leite e derivados, além de produtos cárneos e outros. Por essa razão são necessários estudos de boas práticas de manipulação, higiene e fabricação na indústria de alimentos, a fim de evitar grandes perdas econômicas e graves problemas de saúde humana. Como consequência desses estudos, técnicas de detecção do microorganismo e também de suas toxinas vêm evitando que alimentos contaminados tenham seu processo interrompido ainda durante a cadeia produtiva. Dentre essas técnicas destacam-se as moleculares, as quais, atualmente, têm se mostrado cada vez mais sensíveis e específicas. De maneira geral, a associação de boas práticas de higiene e de técnicas moleculares de detecção são fundamentais para a manutenção da sanidade alimentar, evitando prejuízos na indústria e graves problemas de saúde.

Palavras-chave: Doenças transmitidas por alimentos; Intoxicação alimentar; Toxinas; Boas práticas de manipulação.

Resumen

Las enfermedades transmitidas por alimentos (ETA) afectan a millones de personas en todo el mundo. Son causadas principalmente por bacterias del género *Staphylococcus*, principalmente *Staphylococcus aureus*. Entre estos, algunos producen enterotoxinas que son resistentes a las altas temperaturas y pueden contaminar diversos tipos de alimentos como la leche y los productos lácteos, además de los productos cárnicos y otros. Por ello, son necesarios estudios de buenas prácticas de manipulación, higiene y fabricación en la industria alimentaria para evitar grandes pérdidas económicas y graves problemas de salud humana. Como resultado de estos estudios, las técnicas de detección del microorganismo y también de sus toxinas han estado evitando que los alimentos contaminados tengan su proceso interrumpido durante la cadena de producción. Entre estas técnicas destacan las moleculares, que, en la actualidad, han demostrado ser cada vez más sensibles y específicas. En general, la asociación de buenas prácticas de higiene y técnicas de detección molecular son fundamentales para el mantenimiento de la salud alimentaria, evitando pérdidas en la industria y graves problemas de salud.

Palabras clave: Enfermedades transmitidas por alimentos; Intoxicación alimentaria; Toxinas; Buenas prácticas de manejo.

1. Introduction

One of the biggest health problems worldwide is infection by eating contaminated food, which can lead to foodborne diseases - FBD (Montanhini & Hein, 2013). One of the most common forms of FBD is gastroenteritis caused by consuming food containing microbial toxins. Among food poisoning, the highlights are those caused by *Staphylococcus aureus*, which are responsible for outbreaks of food poisoning worldwide (Goulart et al., 2016).

As the main cause of these intoxications by *S. aureus* we have Staphylococcal Enterotoxins (SEs), which are single-chain and globular proteins with molecular weight ranging between 22 and 29 kDa, soluble in water and in saline solutions (Hennekinne et al., 2013). Many of these are resistant to high temperatures and are not inactivated by heat sterilization processes. In men they are responsible for gastroenteritis, enterocolitis, diarrhea, staphylococcal food poisoning, and other diseases. For these diseases, the severity of the contamination depends on factors such as the amount of contaminated food that was ingested, the amount of toxins present in the food ingested and the patient's previous health status (Sá et al., 2012).

In situations where the bacteria is also present inside the host during the intoxication process, the disease can be aggravated by mechanisms of resistance to antibiotics. Some strains of *S. aureus* can carry antibiotic resistance genes, including *blaZ* and *mecA*. *BlaZ* is a gene that promotes resistance to drugs in the class of β -lactamase-non-stable agents and *mecA* selects resistance to other drugs such as oxacillin and methicillin. The ability to spread this resistance to other microorganisms gives *S. aureus* great importance in the food industry, so that it is not present in the production chain (Aragão et al., 2019).

In view of the harmful effect that contamination of food by *S. aureus* can cause in humans, it is essential that good practices of handling and hygiene are implemented throughout the food industry. In addition to conducting studies of modern techniques able to identify, quickly and specifically, pathogens that cause FBD, thus avoiding economic and health problems.

2. *S. aureus* and its importance in the food industry

2.1 Milk and its derivatives

Milk is a product of animal origin that has a high biological value, being the most complete food in the human diet, especially in the early stages of life (Cruz et al., 2017). It is mainly composed of water, proteins, fats, carbohydrates, minerals, in addition to fractions of vitamins, and therefore ensuring its quality is a matter of public health (Becker et al., 2010).

Contamination by *S. aureus* affects milk composition, either by the extracellular action of enzymes or by the enzymatic mechanisms triggered by the animal's innate immune response to the pathogen (Le Roux et al., 2003). The main changes are in the protein composition, as well as in the milk fat rate. In addition to the decrease in milk production and an increase in expenses with medicines and veterinary assistance (Motta et al., 2011).

In dairy cattle, contamination by *S. aureus* one of the mastitis cause, a condition that promotes an inflammatory process in the mammary gland. Due to its high incidence, mastitis is considered the most relevant disease in dairy cattle and your products (Tozzetti et al., 2008).

In humans, contamination by SEs further gastrointestinal disorders, caused by food poisoning that can occur by ingesting up to 20 ng of the toxin. Ingestion of these SEs can cause food poisoning characterized by vomiting, nausea, diarrhea, abdominal pain, with an incubation period of one to six hours after eating the contaminated food (Hennekinne et al., 2013). The duration of the condition is usually short (24 to 48 hours) and the patient's recovery can occur from one to three days (Holmberg & Blake, 1984).

Order to reduce the high prevalence of *S. aureus* in the dairy industry, a program for the control of microorganisms for milk and dairy products was created, which is based on the correct management of milking (disinfection of post-milking ceilings and milking materials), treatment of all clinical cases, in addition to segregation or disposal of animals with chronic mastitis, for example (Santos & Fonseca, 2006; Denis et al., 2009). Other techniques are shown to be very effective in combating outbreaks of contamination: vaccination (Detillex et al., 2009) and genetic improvement of animals (Ferreira & Simm 2012).

2.2 Meat products

The complex nutritional composition, the high water activity and the pH close to neutrality make beef an excellent medium for the growth of microorganisms. These, in high concentration, can cause deterioration and risk to the consumer's health (Marchi et al., 2012).

Among meat products, ground meat is a product that is easily contaminated with *S. aureus* due to excessive handling (Akbas & Kokumer, 2015). As a result, *S. aureus* can be an important indicator of personal hygiene and the evaluation of industrial sanitation programs (Zhang et al., 2018).

In this type of food, another concern is the formation of bacterial biofilms by *S. aureus* (Fuster-Valls et al., 2008), considering that washing and sanitization may not guarantee the complete elimination of biofilms from this microorganism. Biofilms, when formed, act as sources of constant contamination, releasing cells from the bacteria that constitute them, which can compromise the microbiological quality of food (Lima et al., 2004).

Given the fact that the consumption of meat products is high worldwide, good practices in handling and manufacturing food are very important, in order to have no level of contamination by *S. aureus*, ensuring good quality of food and consequently maintaining consumer health.

3. Safe Food Handling Practices

Due to their ability to inhabit living surfaces, strains of *S. aureus*, throughout human history, have been repeatedly reported for their presence in various food products and also in epidemiological studies of food poisoning outbreaks involving different regions of the world (Erhabor et al., 2019; Liyanage, 2020; Galo et al., 2020). The basis of most reports of food poisoning by *S. aureus* is closely related to undue or scarce good hygiene practices, whether due to the handler's malpractice or negligence, or even to the work environment offered to them (Martin et al., 2004).

In view of this growing global problem, it is seen that during the last few years there has been a continuous cooperation between scientific bodies of great relevance such as the British Standards Institution (United Kingdom) and the Food and Drug Administration (United State of America). Both seeking a consistent exchange of information, based on experiments that improve safe practices in food handling, thus avoiding high numbers of food poisoning. Through these

efforts, these and other organizations were able to issue recommendations for better detection and identification of *S. aureus* and its enterotoxins, in addition to procedures that guarantee food safety, such as Hazard Analysis and Critical Point Control (HACCP) (Martin et al., 2004).

3.1 HACCP System

The HACCP system consists of three stages main points: the first determine the danger to which the consumer may be subjected; the second, identifies the critical control points, ensuring safe management of the danger; and the third, monitoris the the ensure efficiency in the operation of critical control points. This is a well-known system and used in the food industry sector, because through its determinations it is possible to guarantee the food security of the various products sold (Martin et al., 2004).

To ensure this safety, the food industry considers some critical control points, such as:

- Use of raw materials containing minimum levels of *S. aureus*;
- Use of treatments to reduce the microbial load *S. aureus*;
- Use of additives or low temperature to prevent the multiplication of *S. aureus* during handling and storage;
- Use of hygienic handling to prevent the reintroduction of *S. aureus*;

All of these points can be achieved by controlling the factors that influence the proliferation of *S. aureus*, such as temperature, pH, water activity, and sodium chloride concentration in the product (Basti et al., 2007). Constant monitoring, through microbiological analysis, is essential, as there is the possibility that the treatments used to ensure the elimination of the dangers caused by the microorganism are partially effective, since *Staphylococcus* enterotoxins are more resistant to treatments than cells producers of these (Basti et al., 2007).

Thus, the discovery and use of adjuvants to these treatments is necessary and imminent. An adjuvant widely used by scientists are essential oils, as they have a satisfactory efficacy trajectory. This is possible to see through the experiments described in literature (Chen et al., 2008; Ghani et al., 2018).

3.2 Effective treatments against *S. aureus*

3.2.1 Essential Oils

Studies with essential oils are constant, always bringing important results, as in the studies by Ghani and collaborators (2018), who described the effect of the antimicrobial activity of soluble soybean polysaccharide (SSPS) films on fresh meat (Pranoto et al., 2005). Through its results it was possible to detect that SSPS films containing 0.6% nanoemulsion (based on the concentration of cinnamon essential oil) had effects on gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*). SSPS films containing 0.8% nanoemulsion had antimicrobial effects on gram-positive and gram-negative bacteria.

In addition to oils, another adjuvant widely used for food control and safety is antimicrobial peptides, which are fragments of proteins present in the innate immune system of invertebrates and vertebrates as host defense molecules. These can be used on several fronts, mainly in the incorporation at the moment of production of the food itself as well as in the production of its packaging (Damasceno et al., 2014; Santos et al., 2018).

3.2.2 Bioactive Molecules

Active packaging materials containing antimicrobial peptide have emerged as a promising approach to delay food spoilage and increase food security (Pola et al., 2016). One of the main microbial peptides used is nisin, which is produced by the bacterium *Lactococcus lactis*. Using this peptide, aiming at the coating of coalho cheese, the experiment by Damasceno et

al. (2014) generated results that demonstrated the efficiency of the nisin coating, since it inhibited the growth of positive coagulase *Staphylococcus*, in detriment to the control cheese (without coating), increasing its shelf life (Damasceno et al., 2014).

3.2.3 Chemical Treatments

As an alternative to the treatments and adjuvants to control *S. aureus*, a study was recently published using a Citrox solution, composed of 18 g of citric acid, 18 g of malic acid and 5 g of ascorbic acid in 100 ml of water, with a pH of approximately 2.7. Concentrations of 1% and 2% of this solution were used to evaluate the inhibitory potential against methicillin-resistant *S. aureus* (MRSA) in chicken fillet. As a result, the inhibition of MRSA in the chicken fillet was noticed, with the total viable count gradually decreasing in the first three days of storage (Yehia et al., 2020).

The constant discovery of resistance mechanisms by bacteria, requires that science constantly seek new measures of microbial control, especially related to the food industry. When carrying out good hygiene, handling and manufacturing practices associated with the manipulator's day-to-day life, it is critical to reduce food poisoning, in addition to the imminent and constant need for new techniques that detect staphylococcal enterotoxins, in order to avoid the increase in foodborne diseases.

4. Molecular Techniques for Identification of Staphylococcal Endotoxins

In order to diagnose staphylococcal food poisoning, in general, it is necessary to know that SE (Staphylococcal Enterotoxins) are generally detectable in food when the *S. aureus* count exceeds 10^5 CFU / mL or g of food (Wong & Bergdoll, 2002). The biggest problem in identifying SE in food is the small amount that is found (Lancette & Bennett, 2001). To improve the characterization of SE, several techniques have been integrated into the diagnostic and research strategy (Table 1).

Table 1 – Techniques used to identify Staphylococcal Endotoxins (SE).

	Mechanism	Advantages	Disadvantages	Examples	References
Animal testing	Administration of the toxin by gastric or intraperitoneal	First method developed	Low specificity and sensitivity.	Tests on cats and monkeys	(Fulton, 1943)
Skin test	Skin reactions in sensitized subjects	Fast and non-invasive	Low reproducibility	Tests on pigs	(Scheuber et al, 1983)
Serological tests	Antigen/antibody reactions in vitro	Low detection	Semi-qualitative	Gel diffusion/ agglutination test	(Hall, Angelotti & Lewis, 1963)
Molecular methods	Nucleic acid hybridization and PCR	Analysis of strains: toxin genes	High cost and skilled labor	PCR, multiplex, RT-PCR, Real-time PCR (qPCR)	(Neill et al., 1990)
Chromatographic Methods	Peptides analyzed	Quantitative	Difficulty in using protein samples	LC-ESI-MS, LC-MS/MS	(Kientz, Hulst & Wils, 1997)
Immunoassays	Specific monoclonal and polyclonal antibodies	High sensitivity/ Specificity	Reagent complexity.	ELISA RPLA	(Simon & Terplan, 1977) (Park & Szabo 1986)

Source: Authors (2021).

The introduction of molecular biology techniques in microbiology has established a new era in the detection and characterization of microorganisms (Moreira et al., 2018). Molecular methods based on the Polymerase Chain Reaction (PCR)

allow the detection of the nucleotide sequence of the gene, responsible for the production of enterotoxin by staphylococci. They are viable, fast methods widely used in gene research, requiring a small sample and applicable to a wide variety of foods: vegetables, milk, cheese, and meat products (Feitosa et al., 2017). Thus, it is possible to identify factors of pathogenesis, virulence and resistance to antibiotics, providing high sensitivity and specificity (Huertas-Caro et al., 2019).

Several *S. aureus* strains produce SE in food. Five classic SE (*SEA*, *SEB*, *SEC*, *SED* and *SEE*) are the most commonly found. In addition, 16 other types of SE or superantigens (*SEG*, *SEH*, *SEI*, *SEJ*, *SEK*, *SEL*, *SEM*, *SEN*, *SEO*, *SEP*, *SEQ*, *SER*, *SES*, *SET*, *ITS* and *SEV*) have also been described and evaluated, the main genes and targets of several studies are represented by *sea*, *seb*, *sec*, *sed* and *see* (Kim et al., 2011).

In general, conventional PCR is the technique most commonly used in the molecular diagnosis of different food pathogens, developed over 30 years ago (Mahfoozi et al., 2019). In SE analysis, PCR minimizes the time to detect and identify *S. aureus* that is difficult to recover, compared with the methods used previously.

Real-time PCR or Quantitative PCR (qPCR) assays are an evolution of PCR in which the step of evaluating products by agarose or polyacrylamide gel electrophoresis is eliminated. qPCR combines the amplification of specific DNA sequences with fluorophores with DNA affinity or fluorescent labeled probes. Different chemicals have been developed for the tagging of these probes, among the most important we find SYBR Green and hydrolysis probes (TaqMan) (Mahfoozi et al., 2019). DNA sequencing techniques can also be used to confirm gene sequences (Yehia et al., 2019).

Most instrumentation platforms consist of a thermocycler, with an optical system for excitation and collection of fluorescence emission and a computer with its own software for data acquisition and final reaction analysis (Mackay et al., 2007).

Multiplex PCR (PCRm), a conventional PCR variation, uses two or more pairs of primers in the same reaction, allowing the detection of more than one gene simultaneously. In 2017, Mansour and collaborators observed through this technique that strains of *S. aureus* encoded the *see* and *sea* genes in most samples of raw milk, warning of the potential risk of staphylococcal food poisoning.

Reverse transcriptase PCR (RT-PCR) allows expression studies, the amount of transcripts is correlated with the amount of SE produced, so the level of expression of SE genes could be used to assess the toxigenic potential in different isolates. Both PCRm and RT-PCR can be adapted to qPCR and these techniques are now called qPCRm and qRT-PCR, which adds benefits already mentioned above. PCR also has the advantage of being combined with other techniques, such as the most probable number (MPN-PCR) and enzyme immunoassay (PCR-ELISA) which can provide more sensitive results (Gillian et al., 2000).

5. Final Considerations and Perspectives

The presence of *S. aureus* in food, especially in milk and its derivatives, can cause several diseases. These diseases have the potential to cause millionaire losses to the dairy industry, which can cause loss of animals and products, in addition to harming consumers' health. Another industry that is very affected by the action of *S. aureus* is that of meat products, in which the effect of the formation of bacterial biofilm impairs the visual and nutritional quality of the final commercial product.

Advances in studies of more sensitive techniques that detect the presence of *S. aureus* or its enterotoxins in food are of great importance for the prevention of food poisoning outbreaks. Molecular techniques are promising in this detection, combined with maintaining the safety of the food industry and, consequently, human health.

But now? What are we going to expect? New treatments are constantly emerging and, as mentioned before, they tend to continue prioritizing good sanitary practices and biotechnological processes, which then hinder the fixation and growth of microorganisms directly in the body of animal or on the surface of food products. As a result of this global effort, it is expected

that diseases caused by *S. aureus* or their enterotoxins are less frequently associated with different food production chains.

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