Microbial update

Staphylococcus

Produced as a service to the food industry by Oxoid Ltd.

The significance of staphylococcus to the food industry is in the ability of some strains to produce enterotoxins. These enterotoxins are proteins that can cause illness when consumed.

Staphylococcal food poisoning is a worldwide problem. Although its true incidence is unknown, it is one of the main causes of bacterial food poisoning and thus represents a significant burden to the world's economy.

Staphylococcus aureus, the most common strain of staphylococci to cause food poisoning, is a common bacterium found on the skin and in the nose and throat of healthy people and animals. It is also found in the environment and can be present at high levels in unpasteurised milk.

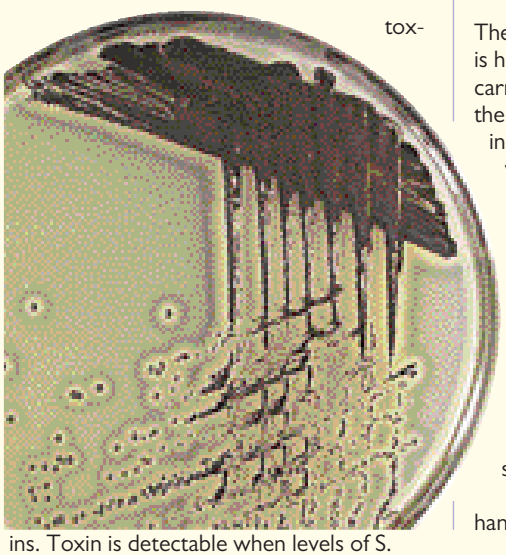
The organism

Staphylococcus aureus is a Gram positive, non-motile, facultatively anaerobic coccus (spherical bacterium). Under microscopic examination, it is characteristically seen in pairs, or bunches, like grapes.

The organism can grow within a temperature range of 7-48°C and a pH range of 4-9.3, although the range for enterotoxin production is narrower.

It is relatively tolerant to salt and is extremely resistant to decreased water activity, demonstrating growth down to an aw value of 0.83. This has implications for the manufacturers of some processed foods.

Some strains of S. aureus (and some other staphylococci) produce heat stable enterotoxins. Toxin is detectable when levels of S. aureus exceed 10^5 cells in a gram of food. Such high levels of S. aureus are, therefore, unacceptable in food products.



S. aureus on Baird Parker +EY.

One of the most commonly used identifying characteristics of S. aureus is the production of coagulase in culture. However, it is now known that some other staphylococci are coagulase positive and that some coagulase negative species are also able to produce enterotoxins. EU regulations stipulate testing for coagulase positive staphylococci rather than only S. aureus.

Food poisoning

Staphylococcal enterotoxins are fast acting. The onset of symptoms can be abrupt, usually within 1-7 hours (but sometimes in as little as 30 minutes) after consumption of the contaminated food. Symptoms include nausea, vomiting, stomach cramps and diarrhoea and are usually short lived, lasting for several hours. The illness is relatively mild, however, and most people recover within 1-3 days.

Cases of staphylococcal food poisoning rarely require hospitalisation, but severe disease may occur in the very young, the very old or the immunocompromised. In such cases, rehydration therapy may be required.

The normally mild nature and short duration of staphylococcal food poisoning may result in under diagnosis or under reporting of the illness, making it difficult to assess its true incidence.

Food processing issues

The main source of contamination in foods is humans, in particular food handlers who carry the bacterium on their hands or in their nostrils. The risk of transmission increases if food handlers have infected wounds or skin infections on their hands or arms, or if they have a respiratory infection that causes them to cough or sneeze. Other routes of contamination include raw materials, such as milk or cheese, or contaminated food preparation surfaces.

Foods that are commonly associated with staphylococcal food poisoning include dairy products, meat and meat products, cream filled bakery products, sandwiches and salads.

The highest risk foods are those that are handled during preparation and do not require cooking. It is possible that some strains of S. aureus may grow and produce toxins in food if subsequent storage is inadequate (for example, if they are left standing at room temperature for more than two hours and eaten cold). It is important to consider the ability of S. aureus to tolerate salt, allowing it to grow in salty foods, such as ham and other cured meats.

Minced meat is one of the foods commonly associated with staphylococcal food poisoning (Shutterstock).

The presence of large numbers of S. aureus, in processed foods may be an indication of inadequate cleaning and/or temperature control. The contamination of foods after they have been cooked or processed, whether by handling or from the environment, presents a significant hazard due to the absence of competitor microorganisms that normally restrict the growth and subsequent toxin production of S. aureus.

S. aureus is destroyed by heat, either through cooking or pasteurisation. The enterotoxins, however, are heat stable and can survive boiling for over an hour. It is possible, therefore, for a well cooked food to cause illness even if it contains no viable S. aureus cells.

Prevention

Due to the widespread presence of S. aureus in humans, the environment and foods, it is important for food manufacturers to follow recommended guidelines for acceptable levels in certain foods. Such criteria are published in the EC Regulation 2073/20053 and are summarised in Table 1.

If results are unsatisfactory, improvements in production hygiene and in the selection of raw materials are recommended.

Demonstration of acceptable levels of S. aureus in foods should never replace good

preventative measures.

Staphylococcal food poisoning can be avoided by preventing foods from being contaminated with S. aureus and/or preventing its subsequent growth to levels that result in toxin production. Emphasis must be placed, therefore, on good hygiene procedures and temperature control systems.

Contamination from food handlers can be minimised by following measures, such as:

- Thorough hand washing.
- Excusing staff with visible infected wounds or skin infections on their hands or arms.
- Excusing staff with respiratory infections.
- Wearing appropriate clean clothing, such as hats, gloves and gowns.

In order to prevent contamination from the environment it is important to ensure that all food preparation surfaces are cleaned and disinfected adequately.

Furthermore, to prevent any S. aureus present in foods from growing to significant levels, food should be stored appropriately prior to consumption.

The potential for enterotoxin production is greater in foods that are left to stand at temperatures that allow S. aureus to grow. Cold food should, therefore, be stored at temperatures less than 7°C and hot food should be stored at temperatures greater than 60°C prior to consumption.

It is also necessary to store raw foods appropriately in order to control microbial growth and the production of heat stable enterotoxin prior to cooking.

Reading results for Staphylococcus aureus on the Oxoid BAX system.

Methods of detection

Culture is the principal means of detecting staphylococci in foods. Such methods for the growth and detection of S. aureus and other coagulase positive staphylococci may indicate if a food is at risk of staphylococcal enterotoxin contamination.

High risk foods, such as raw milk or unpasteurised dairy products, may contain high numbers of the organism. Environmental samples or processed foods, on the other hand, may contain fewer bacterial cells and therefore may require enrichment prior to detection and enumeration.

In many instances, S. aureus may not be the predominant species and so a selective growth medium is required to favour the growth and detection of this species.

Enrichment

Small numbers of S. aureus in foods may not present a risk to humans, depending on subsequent processing and storage conditions. However in some products, such as dried milk and infant foods, small numbers may multiply rapidly following reconstitution, thus presenting a health risk to the consumer.

In such circumstances, samples for analysis may require selective enrichment in an appropriate broth, such as Giollitti-Cantoni Broth or Baird-Parker Liquid Medium. Both of these media contain potassium tellurite as an indicator.

As a result, the growth of S. aureus causes blackening of the broth, making the identification of positive samples easier.

Enumeration

A wide range of selective culture media have been developed for the isolation and enumeration of S. aureus and other coagulase positive staphylococci, many of which have been adapted to suit particular food types.

The media recommended in BS EN ISO 6888 parts 1 and 2 for the enumeration of coagulase positive staphylococci are Baird-Parker Agar with Egg Yolk Tellurite (BP-YET) and Baird Parker with Rabbit Plasma Fibrinogen Agar (BP-RPF).

On BP-EYT, typical colonies appear grey/black with a clear zone (or halo) around the colony. This is a presumptive identification which must then be confirmed.

The usual confirmatory test for S. aureus is demonstrating the presence of coagulase. This can be performed in a test tube (the coagulase tube test) or by using a rapid haemagglutination test, such as Oxoid Staphylase, or a latex agglutination test, such as Oxoid Staphytect Plus. The latter also detects Protein A, which is found on the surface of S. aureus, making it highly specific for this micro-organism.

On BP-RPF, coagulase positive staphylococci are generally a paler grey colour, due to reduced tellurite content, with an opaque halo of fibrin precipitation, indicating coagulase activity. Colonies displaying these typical characteristics do not require further confirmation.

In infant formula, small numbers of S. aureus may multiply rapidly following reconstitution, thus presenting a health risk (Shutterstock).

Rapid detection methods

Methods for the cultural detection of S. aureus may take 2-6 days to obtain a result. For some sensitive or ready-to-eat foods, such delays may be problematic.

It is now possible to detect specific sequences of bacterial DNA quickly and routinely using methods such as polymerase chain reaction (PCR). Simple methodology and automation has enabled this method to

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Table 1. Criteria for the presence of coagulase positive staphylococci in certain food types.

Food type	Criteria (cfu/g) Satisfactory	Acceptable	Unsatisfactory
Raw milk cheese	$<10^4$	2/5 samples between 10^4 and 10^5	$>10^5$
Unpasteurised cheese/ ripened pasteurised cheese	$<10^2$	2/5 samples between 10^2 and 10^3	$>10^3$
Unripened pasteurised cheese	<10	2/5 samples between 10 and 10^2	$>10^2$
Milk and whey powder	<10	2/5 samples between 10 and 10^2	$>10^2$
Shelled and shucked cooked shellfish	$<10^2$	2/5 samples between 10^2 and 10^3	$>10^3$

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be performed in routine laboratories and systems, such as the DuPont Qualicon BAX System Q7 (available from Oxoid in Europe, Australia and Canada), are now widely used to detect a broad range of foodborne pathogens, including S. aureus.

The BAX System Real-Time assay for S. aureus provides positive or negative results within just 24 hours. Although it does not enumerate the organism, a threshold result can be achieved by utilising an initial dilution of sample.

Toxin detection

Not all strains of S. aureus produce enterotoxins and so the presence of the organism alone is not sufficient evidence that it is responsible for an outbreak of food poisoning. For this, it is necessary to demonstrate that the isolated organism produces toxin, or that toxin is present in the food product.

Furthermore, the absence of viable S. aureus cells does not necessarily mean that the food is free from staphylococcal enterotoxins, since processing conditions may have destroyed the organism while the toxin remains, as discussed previously.

A number of methods for detecting enterotoxins in foods are available. These methods include the microslide method and immunological techniques, such as radioimmunoassays (RIA) and enzyme linked immunosorbent assays (ELISA).

For demonstrating the production of toxin in S. aureus isolates, membrane-over-agar, sac culture and semi-solid agar techniques have been used. In addition, immunological methods such as reversed passive latex agglutination (RPLA) are available.

Protecting consumers

It is almost impossible to completely eliminate the presence of S. aureus in our foods and in the environment, however it is possible to ensure that levels do not reach the threshold where toxin production is likely to cause illness.

Strict hygiene procedures and proper storage of foods prior to consumption is of paramount importance. It is also important to monitor the effectiveness of such measures with regular microbiological testing, where appropriate. As testing methods continue to develop and provide new levels of speed, accuracy and sensitivity, manufacturers will have greater peace of mind – assured that they have made every effort to protect consumers from staphylococcal food poisoning. n

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