6. Staphylococcus aureus

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6 Staphylococcus aureus

6.1 General information

General information

S. aureus is a Gram-positive, spherical bacterium (coccus) with a diameter of 1 – 1.3 µm. When viewed microscopically, *S. aureus* appears in clusters, like bunches of grapes. Growing in food, some strains can produce toxins which cause acute gastro-intestinal diseases if ingested. The enterotoxin produced by *S. aureus* is a heat-stable protein, which survives heating at 100 °C for 30 – 700 minutes.

Occurrence

The main reservoirs of *S. aureus* are humans and animals. Healthy people carry the organism in their nose and throat (50 %), on their hands (5-30 %), and in wounds. *S. aureus* can also colonise food contact surfaces, and it can become a persistent organism in slaughterhouses. *S. aureus* can contaminate foods through contact with contaminated hands, materials and surfaces, but also via the air (coughing).

Taxonomy

S. aureus is a Gram-positive, enterotoxin producing organism. Together with other species, such as *S. intermedius*, *S. hyicus* and *S. epidermidis*, *S. aureus* belongs to the genus *Staphylococcus*. *S. aureus* can be distinguished from *S. epidermidis* by the production of the enzymes coagulase and thermonuclease. Not only does it produce enterotoxin, which causes food poisoning when ingested, the organism also causes a number of other diseases, e.g. wound infections and blood poisoning (sepsis), toxic shock etc.

On a rich medium, *S. aureus* forms fairly large, yellow colonies. The organism can grow both with and without oxygen (facultatively anaerobic), and is catalase-positive and oxidase-negative. Virtually all *S. aureus* strains produce the enzyme coagulase.

Figure 1 shows an electron microscope image of *S. aureus.*

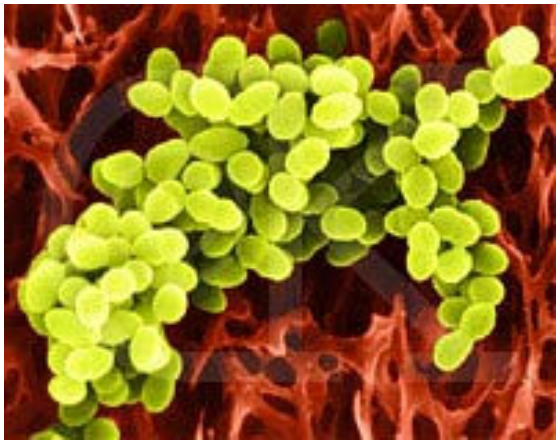

Figure 1. Electron microscope image of *S. aureus.* The clusters of cocci, resembling bunches of grapes, are clearly visible on the background surface. *S. aureus* cells do not possess flagellae (image produced by the University of Warwick, USA).

Growth

S. aureus can grow both aerobically and anaerobically in various foods. It is characteristic that staphylococci can grow at low water activity (approx 0.86), corresponding with a salt content of about 14 %.

Table 1. Limits of growth for *S. aureus*

Parameter	Reported values
Temperature	
Minimum temperature	8 ºC
Optimum temperature	35 – 37 ºC
Maximum temperature	45 ºC
Water activity (a_w)	
Minimum a_w	0.86 – 0.84
pH	
Minimum pH	4.5
Optimum pH	7.0 – 7.5
Maximum pH	9.3

Survival

Table 2 presents data concerning the survival and heat resistance of S. aureus.
S. aureus is extremely heat sensitive. It is already inactivated at a temperature > 46 ºC.

Table 2. Survival and heat resistance of *S. aureus*

Survival	*Time*
Frozen products	Years
Dry products	Weeks, up to a few months
Resistance to heating	
Heat $D_{50.0}$ (0.1 M phosphate buffer)	9.5 – 42.2 min.
Heat $D_{55.0}$ (0.1 M phosphate buffer)	3 min.
Heat $D_{62.8}$ (NaCl solution)	0.4 – 1.1 min.
Resistance of toxin to heating	
Heat D_{100} (milk)	70 min.
Heat D_{110} (milk)	26 min.
Heat D_{120} (milk)	9.4 min.

Illness and symptoms

Illness is caused by the toxin which *S. aureus* has produced in the foodstuff. In order to produce detectable levels of toxin, the number of organisms must be over 10^{5-6} per gram of product. The

time between ingestion of the toxin and the symptoms is only two to five hours and depends on the amount and type of food and the state of health of the person. The main symptoms are:

- nausea;
- vomiting;
- abdominal cramps;
- exhaustion.

Patients usually recover within two days. No more than just 1 µg of toxin is enough to cause illness. This level is already reached when just over 10^5 *S. aureus* organisms are present per gram of food.

The diagnosis is based on the clinical symptoms in combination with analysis of the suspect food. The presence of large numbers of *S. aureus* in the food is an indicator for the presence of enterotoxin. The final proof is delivered by detection of the toxin, notably in products that have been heated before consumption.

Food products associated with *S. aureus*

Foods frequently involved in foodborne intoxications caused by *S. aureus* are:

- meat and meat products;
- poultry meat and egg products;
- egg salads, fish, poultry meat, potatoes and pasta;
- pastry like cream and custard cake;
- dairy products.

What these products have in common is that they require frequent manual handling during preparation and that they are often kept at room temperature for some time.

Prevention

Most food intoxications caused by *S. aureus* are the result of bad hygienic practices in household and industrial kitchens. The largest risk occurs during the preparation of products that are contaminated by *S. aureus* after heating. If cooling of these contaminated foods is slow or insufficient, *S. aureus* can multiply to large numbers and produce toxin in the food. If the food is heated before consumption, *S. aureus* organisms will be inactivated, contrary to the toxin, which is heat stable and will cause food intoxication.
The principal preventive measures include:

- storing raw food products in the refrigerator;
- preventing cross-contamination between raw and prepared products;
- cooling and storing prepared products at a temperature < 7 °C;
- careful cleaning of hands and contact surfaces prior to food preparation;
- washing and cleaning kitchen utensils with a hot detergent solution after contact with raw food products;

Incidence

There are various reasons why the actual incidence of *S. aureus* food poisoning is unknown, for instance: the illness has not been diagnosed or not been reported, or the wrong food is analysed.

In the Netherlands, the number of intoxications is estimated at 10,000 to 50,000 cases per year. Twenty-three outbreaks were registered between 1993 and 1998, involving 95 persons in total. During the same period, thirteen individual cases were reported.

Death following a case of *S. aureus* food intoxication is very rare. It is assumed that all persons are sensitive to this bacterial intoxication, but the symptoms and the severity may vary per individual.

6.2 Legislation

S. aureus enterotoxins must be absent from ready-to-eat products.

Food safety criteria for enterotoxin have been laid down in *Commission Regulation (EC) N°2073/2005 on microbiological criteria for foodstuffs* only for cheese, milk powder and whey powder. These products are presented in Table 1. In addition, process hygiene criteria have been laid down for the number of coagulase-positive staphylococci *(S. aureus)* allowed in food products during the production process. These criteria are listed in Table 2.

Table 1. Food safety criteria for enterotoxins of coagulase-positive staphylococci [1,2)]

Food category	Sampling scheme [2)]		Limit	Stage where the criterion applies
	n	c		
Cheese, milk powder and whey powder, as referred to in the process criteria for coagulase-positive staphylococci [3)]	5	0	Absent in 25 g	Products placed on the market, during their shelf life

1) For details consult *Commission Regulation (EC) N°2073/2005 on microbiological criteria for foodstuffs*.
2) The toxin must be analysed using the reference method of the Community Reference Laboratory, or another equivalent method.
3) n = number of units comprising the sample; c = number of sample units in which enterotoxin is allowed be present
4) See Table 2

If the criteria are not met, the batch of product is not to be placed on the market. Products that have been placed on the market must be withdrawn or recalled. For details concerning withdrawal and recall of products consult *Commission Regulation (EC) N°2073/2005 on microbiological criteria for foodstuffs*.

Table 2. Process hygiene criteria for coagulase-positive staphylococci [1)]

Food category	Sampling scheme [2)]		Limit [2)]		Stage where the criterion applies
	n	c	m	M	
Milk and dairy products					
Cheese made from raw milk	5	2	10^4 cfu/g	10^5 cfu/g	At the time during the production process when the number of staphylococci is expected to be the highest
Cheese made from milk that has undergone a lower heat treatment					At the time during the

than pasteurisation (7) and ripened cheeses made from milk or whey that has undergone pasteurisation or a stronger heat treatment	5	2	10^2 cfu/g	10^3 cfu/g	production process when the number of staphylococci is expected to be the highest
Unripened soft cheese (fresh cheese) made from milk or whey that has undergone pasteurisation or a stronger heat treatment	5	2	10 cfu/g	10^2 cfu/g	End of the production process

[1] For details consult *Commission Regulation (EC) N°2073/2005 on microbiological criteria for foodstuffs*.

[2] *S. aureus* must be analysed using the reference method (EN/ISO 6888-1 and –2), or another equivalent method.

[3] n = number of units comprising the sample; c = number of sample units giving values between m and M.

If the results of testing against the process hygiene criteria are unsatisfactory, the production hygiene must be improved. If values > 10^5 cfu/g are detected, the batch has to be tested for staphylococcal enterotoxins.

6.3 Examples from practice and research

6.3.1 Foodborne intoxications

Foodborne intoxications caused by Staphylococcus aureus

In September 1997, an outbreak of food poisoning occurred in Florida (USA) after consumption of ham contaminated with *S. aureus* enterotoxin. The report of this outbreak was published in *Morbidity and Mortality Weekly Report* (MMWR). Thirty-one persons of a group of 125 participating in a party fell ill, the symptoms being nausea (94 %), vomiting (89 %), diarrhoea (72 %), sweating (61 %), cold shivers (44 %), fatigue (39 %), muscular aches (28 %), headache (11 %) and fever (11 %). The symptoms developed three to six hours after consumption of the ham, and lasted for about 24 hours. Seven persons requested medical attention and two of them were admitted to hospital for further treatment.

The cause: The day before the party, a pre-cooked and packed ham of about 8 kg was bought and roasted in an oven for 1.5 hours at 204 °C. After roasting, the hot ham was sliced with a commercial slicer, which had not been cleaned prior to slicing. The sliced ham was placed in a plastic container covered with foil, and stored for six hours in the entry of a cold store. The next day, the ham was served cold.

Source:
MMWR, 46, No. 50, 1997.

Editorial note: The ham had probably become contaminated with *S. aureus* during slicing. Due to the fact that the ham was sliced hot and stored in such a way that cooling was delayed, *S. aureus* could multiply rapidly and produce enterotoxin.

Contaminated Schwarzwalder Schinken

In 1996, the Robert Koch Institute (Wernigerode, Germany) reported a large outbreak of food poisoning, caused by consumption of Schwarzwalder Schinken. The ham (at least six different batches) appeared to be contaminated with *Staphylococcus aureus* enterotoxin. Further investigations led to the following conclusions:

- there was a general lack of hygiene at the production plant;
- the ham from this plant appeared to be heavily contaminated with *S. aureus*;
- the majority of the isolated *S. aureus* strains produced enterotoxin;
- the isolated *S. aureus* strains belonged to five clonal types;
- the greater part of the ham was contaminated with one single clonal type of *S. aureus*.

Source:
Report of the Nat. Reference Centre for Staphylococci at the Robert Koch Institute, Wernigerode, Germany.

Massive Staphylococcus aureus *food poisoning in Japan*

Late June 2000, Japanese consumers fell ill after drinking milk. On 30 June 2000, 1152 patients had reported ill, with vomiting, nausea and diarrhoea as the main symptoms. On 6 July, the number of patients had risen to 10,780, and 159 patients had been admitted to hospital for treatment. On 7 July, the reported number of patients had increased to 12,928, and on 11 July to 14,000. A total of 14,555 persons were reported ill.

Epidemiological research revealed that milk from Snow Brand Food Co Ltd, Japan's biggest dairy company, was the source of the intoxication. Laboratory analysis showed that *Staphylococcus aureus* enterotoxin was present in a number of packages of milk. Further investigation of the manufacturing plant revealed rather poor hygiene standards. The two chief infringements were:

1) Use of loose pipe connections, not included in the automatic cleaning and disinfection system. Large numbers of *S. aureus* organisms were detected in these pipes. Some pipes had not been cleaned for three weeks.
2) Reworking of returned milk. Packages of returned milk were opened by hand and stored in a separate tank which could not be cooled and which was not included in the automatic cleaning and disinfection system. The return milk was mixed with raw milk, and packed after pasteurisation.

It was later confirmed that the *S. aureus* strains isolated from the pipes produced the same toxin (enterotoxin A) that was present in the milk.

Source:
FoodSafetyNet Period July-August 2000.

Editorial note: Initially, it was not clear in which way the milk had become contaminated with the *S. aureus* enterotoxin. *S. aureus* only produces toxin at temperatures that are higher than the usual storage temperature of 8-10 °C. When it became apparent that the return milk was reworked illegally, and that the pipes and storage tank were not included in the automatic cleaning and disinfection system, it became clear that the producer had made gross errors. And when they found out that packages of return milk had been opened by hand, and that the storage tank had not been cooled adequately, the picture was complete.

Food poisoning in Australia

Between March and April 2002, an outbreak of food poisoning occurred in Australia, in which more than 250 people were involved. At the end of March, about 600 people participated in a service in the Imam Ali Islamic Centre in Victoria. After the service, a meal was served consisting of rice, lamb and potatoes. A number of people consumed the food on the spot, while others took the food home.

Health authorities investigating on the spot reported that the meals had been prepared the day before and had been heated before consumption. The main symptoms were acute abdominal cramps, vomiting and nausea. More than 100 patients had to be treated in hospital for

dehydration. The authorities collected remnants for further analysis. The participants who had taken the food home had to be warned and were asked not to consume the food.

Source:
ProMED-mail post, 26 March, 2002. (www.promedmail.org)

Editorial note: Considering the way of preparation, the way of storing the food overnight and the clinical symptoms, the most likely cause of the complaints is poisoning. This may have been caused by enterotoxin produced by *Staphylococcus aureus*. Another possibility is the contamination of food with the emetic toxin which is produced by *Bacillus cereus*. Both toxins are heat stable. Considering the severity of the symptoms, poisoning with *S. aureus* enterotoxin is the most obvious cause.