

Staphylococcus aureus and staphylococcal enterotoxins

Family: Staphylococcacceae Genus: Staphylococcus

Bacterium

Characteristics and sources of *Staphylococcus aureus* and staphylococcal enterotoxins

Main microbiological characteristics

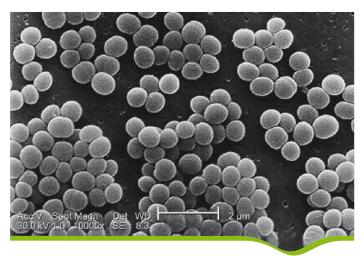
human foodborne illness is an intoxication caused by the ingestion of staphylococcal enterotoxins (SEs), heat-resistant proteins preformed in food in which SE-producing *S. aureus* (or any other staphylococcus) has been able to develop and produce its toxins.

S. aureus is a Gram-positive coccal bacterium, measuring from 0.5 to 1 µm in diameter. It is non-spore-forming, non-motile, facultatively anaerobic and catalase- and coagulase-positive. S. aureus, the type species of the genus Staphylococcus, also commonly called "golden staph", produces numerous toxins including SEs, which are produced by certain S. aureus (those carrying the genes for these toxins) and are responsible for outbreaks associated with this bacterium. To date, 21 different serotypes (SEA to SEE, SEG to SEV) have been described. Only six of them have been clearly demonstrated to have been involved in cases of intoxication: SEA (the most frequently detected serotype in intoxication) to SEE, and SEH. However, since the emetic nature of the SEG, SEI, SER, SES and SET toxins has also been demonstrated, they should be taken into account when characterising toxic episodes. Other species of coagulase-producing staphylococci can also produce SEs, specifically S. intermedius, but their role in intoxication cases to date remains poorly documented. Moreover, S. aureus is the species of coagulase-producing staphylococcus found most frequently in foods.

Table 1. Survival, growth and toxinogenesis characteristics of *S. aureus*

	Gro	wth	Toxins (SEs)		
Parameters	Optimum	Extremes*	Optimal production		
Temperature (°C)	35-41	6-48	34-40	10-45	
рН	6-7	4-10	7-8	5-9.6	
a _w	0.99	0.83-0.99	0.99	0.86-0.99	
NaCl (%)	0-4	0-20	0-4	0-10	
Atmosphere	aerobic	aero- anaerobic	aerobic	aero- anaerobic	

^{*} Extreme values given in the table have only been observed for some strains.



Staphylococcus aureus (SEM) © CDC/Matthew J. Arduino, DRPH

Hazard sources

SEs are proteins produced by coagulase-producing staphylococci, mainly *S. aureus*. These toxins, if present in food in sufficient quantities, can trigger the symptoms of intoxication (Table 2). Staphylococci are ubiquitous bacteria found on the skin, mucous membranes and nasopharyngeal area of warm-blooded animals (mammals, birds), and particularly humans. Coagulase-producing staphylococci are responsible for clinical and subclinical mastitis in cows and other ruminants.

These bacteria can also be isolated from the natural environment (soil, fresh and sea water, dust, air), the domestic environment of humans (kitchens, fridges), the hospital environment and food preparation facilities, as well as from food. As human and animal skin and mucous membranes can harbour *S. aureus*, its presence in the environment is likely due to contamination by humans or animals.

Transmission routes

Apart from cases contracted in the laboratory, all the cases of SE intoxication described to date have been foodborne. Contamination of food is mostly of human origin. It may occur either by direct or indirect contact (contaminated squamae, droplets from the respiratory tract containing the micro-organism).

Contamination of food can also be of animal origin, the most common being the contamination of milk from cows with mastitis.

S. aureus can also cause infections which are sometimes fatal (paronychia,

Recommendations for primary production

 Preventive measures in primary production should include the monitoring of animal health (e.g. mastitis), good handling practices, cleaning and disinfecting of equipment and premises, as well as strict hand washing and the wearing of a cap.

Human foodborne illness

Nature of the disease

The characteristics of the disease are presented in Table 2.

The duration of the incubation period and the severity of the symptoms depend on the amount of enterotoxins ingested and the susceptibility of each individual.

Susceptible population groups (1): no particularly susceptible population.

Dose-effect relationships(2)

Although several studies have attempted to assess the minimum dose to be ingested that triggers the first symptoms, this remains unclear. Epidemiological studies conducted following two incidents involving SEA and affecting a large number of patients reported that the ingestion of doses of between 20 and 144 ng triggered the symptoms in the exposed population.

Epidemiology

Cases of staphylococcal intoxication are monitored through the mandatory reporting of food poisoning outbreaks (FPOs). Note that many outbreaks, particularly those occurring within families, are certainly unreported or undiagnosed.

The French Institute for Public Health Surveillance (InVS) reported that S. aureus may have played a role in 17.9% of outbreaks in France in 2009. The pathogen was confirmed in 34 outbreaks (165 cases, 45 hospitalisations) and suspected in 191 others (1,558 cases, 163 hospitalisations, 3 deaths). Thus in terms of the number of outbreaks, S. aureus was the leading bacterial agent responsible for FPOs. Of these 225 outbreaks, 63.1% of the staphylococcal food poisoning outbreaks (SFPOs) occurred in mass catering and 29.3% in family situations. The FPOs occurring in mass catering were responsible for 79.6% of cases. This percentage difference shows the predominance of mass catering in the number of outbreaks and consequently in the number of sick people. This inequality is partly explained by the very high reporting rate for cases occurring in mass catering.

In Europe, in 2009, S. aureus was the third agent (after Salmonella spp. and enteric viruses) responsible for foodborne illnesses, accounting for 10.1% of outbreaks. S. aureus was implicated in 293 outbreaks corresponding to 2,671 cases, 303 hospitalisations and 3 deaths. The pathogen was confirmed in 88 outbreaks (978 cases, 165 hospitalisations, 2 deaths) and suspected in 205 others (1,693 cases, 138 hospitalisations, 1 death). Its incidence can vary considerably depending on the surveillance system set up in each Member State. For example, in 2009, of the 18 Member States reporting SFPOs, France accounted for 75% of these notifications. Finally, many authors consider that staphylococcal intoxications are a major cause of foodborne illness throughout the world.

The main foods implicated in SFPOs are cooked dishes. SE-producing staphylococci are the leading cause of FPOs involving milk and dairy

Role of foods

Main foods to consider

Four conditions are required for food to be contaminated by S. aureus and its enterotoxins:

- a source of enterotoxin-producing staphylococci (raw food materials, healthy or sick carrier, etc.);
- a means of transmission to the food (contaminated cutting tool, healthy or sick carrier, etc.);
- · a food whose physico-chemical characteristics favour the growth of S. aureus and toxinogenesis (see the foods most "at risk", below);
- a favourable temperature for the period required for significant bacterial multiplication and toxinogenesis.

Table 2. Characteristics of intoxication due to the ingestion of staphylococcal enterotoxins

Mean incubation period	Target population	Main symptoms	Duration of symptoms	Duration of the contagious period	Complications	Carriage of SE-producing staphylococci
30 min - 8 (3 h on average)	Entire population, all age groups combined	Nausea followed by characteristic uncontrollable vomiting (projectile vomiting) Abdominal pain Diarrhoea Dizziness Chills General weakness sometimes accompanied by mild fever In severe cases, headache, prostration and hypotension have been reported.	18 - 24 h Diarrhoea and general weakness may last a further 24 hours.	Enterotoxins non-communicable from person to person. No contagiosity.	Mortality remains exceptional (mortality rate: 0.02%), affecting those most sensitive to dehydration (infants and the elderly) and people with underlying disease. Estimated hospitalisation rate: 16% in cases where the agent was confirmed.	Intestinal carriage: 20-30% of adults Nasal carriage: 20-55% (37% on average) of adults Manual carriage: 10% of hospital staff or staff of a restaurant chain

⁽¹⁾ Susceptible population group: people with a higher than average probability of developing symptoms of the disease, or severe forms of the disease, after exposure to a foodborne hazard [definition used in ANSES data sheets].

⁽²⁾ Relationship between the dose (the quantity of microbial cells ingested during a meal) and the effect on an individual.

S. aureus can be isolated from a wide variety of foods. The foods most "at risk" are:

- foods recontaminated following heat treatment or any other process for eliminating commonplace microflora. The more the food is handled, the greater the risk is high. These include foods such as poultry meat, cooked sliced ham, mixed salads (including rice or vegetable salads), cream cakes and cooked meals handled after cooking;
- foods fermented by slow acidification allowing the growth of *S. aureus* during fermentation. For example, some cheeses and some fermented cured meats such as salami. The risk is increased if the ferments used to initiate production are insufficient, or if none have been added;
- products that are dried or have reduced water content, in which the growth of S. aureus may have been facilitated at one of the manufacturing or storage steps due to a reduced aw and a favourable temperature. These foods include powdered milk, pasta, dried fish.

Dishes requiring human handling (mixed salads, cooked dishes) and dairy products were the foods most frequently associated with staphylococcal intoxications reported in Europe and France in 2009.

Inactivation treatments in industrial environments

Table 3. Inactivation treatments of S. aureus in foods

Disinfectants	Effects of temperature			
S. aureus is sensitive to all disinfectants authorised in the food-processing sector, on condition that the recommended procedures for use are followed.	S. aureus can be destroyed by pasteurisation. Values of D* (for foods with an a_w of 0.99 and a pH between 6.5 and 7), varies depending on the food and the strain of S. aureus tested $D_{60^{\circ}C} \qquad 0.8 - 10 \text{ min}$ $D_{72^{\circ}C} \qquad 0.1 - 1 \text{ s}$			
Irradiation	High pressure			
D_{10} - value** = Cy	Poultry meat: 600 MPa for 15 min at 20°C → 3 log ₁₀ reductions. Marinated beef (a _w = 0.985): 600 MPa for 6 min at 31°C → 2.67 log ₁₀ reduction. Cooked ham (a _w = 0.89): 600 MPa for 6 min at 31°C → 1.12 log ₁₀ reduction. Dried ham (a _w = 0.89): 600 MPa for 6 min at 31°C → 0.55 log ₁₀ reduction.			

- * D is the time needed to divide by 10 the initial population of a microbiological hazard.
- ** D_{10} value is the dose of radiation required to reduce the viability of a population by 90% (1 \log_{10} cycle) under the stated conditions.

Unlike the bacterium, SEs are stable under the heat treatment conditions usually applied to foods. Depending on the amount of enterotoxins initially present, sterilisation (of milk for example, by UHT treatment) may cause a partial denaturation of the SEs, leaving enough in the food to cause an intoxication. In addition, SEs are resistant to most treatments applied in the food industry. It must therefore be assumed that once formed in the food, the enterotoxins cannot be sufficiently inactivated to eliminate the risk of intoxication.

Monitoring in food

Regulation (EC) no. 2073/2005, as amended, on microbiological criteria for foodstuffs, sets:

- a process hygiene criterion for coagulase-producing staphylococci (CPS)⁽³⁾ in milk and milk products and in shelled and shucked products of cooked crustaceans and molluscan shellfish;
- a safety criterion for staphylococcal enterotoxins in milk and milk products in the event of failure to respect the hygiene criterion.

CPS are counted according to the French NF EN ISO 6888 standard $^{(4)}$ - parts 1, 2 and 3. The first two parts are conventional techniques for counting colonies on selective agar medium after direct inoculation of

decimal dilutions of the food and incubation at 37°C. The third part describes a screening and counting method using the most probable number technique after an enrichment step. Staphylococcal enterotoxins types SEA to SEE are detected according to the method of the European Union Reference Laboratory for CPS including *S. aureus*, using qualitative immunochemical tools. To date, commercially available kits are only able to detect toxins SEA to SEE.

Recommendations to operators

- Equipment and premises must be scrupulously cleaned and disinfected, given the strong adherence of staphylococci to surfaces.
- To account for the fact that a great many operators are healthy carriers, the cleaning and disinfecting of hands and the wearing of a cap which covers the hair entirely are good hygiene practices (GHPs) that must be followed.
- In addition, food handlers with skin lesions should be excluded from the handling of unpackaged food, unless the lesions are properly covered (by wearing gloves). Similarly, if any nasopharyngeal symptoms are observed, masks must be worn. Given the high proportion of healthy carriers, and the fact that carriage is not constant in most individuals, screening for *S. aureus* during medical visits is needless. Contamination is prevented through rigorous application of the GHPs mentioned above.
- As these measures are not sufficient to achieve a zero rate of infection, the staphylococci must be destroyed by appropriate treatment (thermal or other), before they can multiply, or their multiplication must be prevented by maintaining food below 6°C. Respecting the cold chain is therefore vital with regard to staphylococci.
- Any technological food processes applied in a hazardous temperature zone (from +10°C to +45°C) must be of short duration or based on parameters other than temperature to stop the growth of the bacteria, such as a pH below 5.
- As staphylococci are heat sensitive whereas their enterotoxins are heat stable, decontamination of a product heavily contaminated with S. aureus cannot be guaranteed by a heat treatment. This will destroy the bacteria but not their enterotoxins, if present. Once formed in the food, the enterotoxins cannot be effectively eliminated.

Domestic hygiene

Recommendations for consumers

- Good hygiene practices should be followed when handling and preparing food (hand washing, cleaning equipment, etc.).
- Before preparing meals, disinfect and protect any wounds or pus-filled pimples with a waterproof dressing, or use gloves.
- It is essential to respect the cold chain and to refrigerate cooked food as soon as possible, if not consumed immediately.

 $[\]textbf{(3) S. } \textit{aureus} \textbf{ is the species of coagulase-producing staphylococcus found most frequently in foods.} \\$

⁽⁴⁾ Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species).

References and links

General references

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Useful links

- ANSES: www.anses.fr
- EFSA: www.efsa.europa.eu
- European Union Reference Laboratory (http://www.ansespro.fr/eurl-staphylococci/) and National Reference Laboratory (NRL) for coagulase-positive staphylococci including Staphylococcus aureus and staphylococcal enterotoxins: ANSES Maisons-Alfort Laboratory for Food Safety.
- French Institute for Public Health Surveillance (InVS): http://www.invs.sante.fr/surveillance/tiac
- National Centre of Reference (NCR) for staphylococci: Hospital Group
 of East, Centre of Microbiology and Pathology, Institute of Microbiology,
 Claude Bernard University, Lyon-I (Lyon).