Staphylococcus aureus

(Material from Maha Hajmeer)

History
1878 – Koch observed staphylococci.

1880 – Pasteur observed staphylococci.

1881 – Ogston named staphylococci.

1884 – Rosenback grew staphylococci on a solid medium.

1884 – Sternberg associated staphylococci with “ptomaine” formation in cheese that caused human illness.

1894 – Denys associated illness with consumption of meat from a cow sick with pyogenic staphylococci.

1907 – Owen recovered staphylococci from dried beef that had caused poisoning characteristic of what now is called staphylococcal food poisoning.

1914 – Barber was the first scientist to relate staphylococcal food poisoning to a toxic substance produced in food. He isolated staphylococci from contaminated milk that came from a cow with mastitis.

1929 – Gail Dack rediscovered the role of staphylococci in food poisoning. He studied an outbreak of food poisoning caused by an X-mas cake. He showed with human volunteers (starting with himself) that the isolated staphylococci produced a toxic substance in culture, this substance caused typical staphylococcal food poisoning.

Since 1948 – A number of studies demonstrated the presence of preformed enterotoxin in foods that had caused staphylococcal food poisoning.

1974 – Josefczyk demonstrated antitoxin in the blood of people that had suffered staphylococcal food poisoning.

Best estimates of annual cases and deaths, US — (Mead et al., 1999).

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Percent</th>
<th>Deaths</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>185,060</td>
<td>1.3</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>All bacterial</td>
<td>4,175,565</td>
<td>30.2</td>
<td>1,297</td>
<td>71.7</td>
</tr>
<tr>
<td>All foodborne</td>
<td>13,814,924</td>
<td>100</td>
<td>1,809</td>
<td>100</td>
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</tbody>
</table>
Contemporary problems
Foods associated with staphylococcal food poisoning:
   In USA: meat products such as ham and desserts
   In Japan: rice balls
Seasonal variation:
Highest incidence of *S. aureus* poisoning during summer months

Name of illness and causative agent
Under favorable growth conditions, *S. aureus* causes foodborne intoxication known as *Staphylococcus*/staphylococcal food poisoning. This poisoning is one of the most common types of foodborne diseases worldwide.

Since the microorganism causes foodborne intoxication rather than infection, the thermostable enterotoxins and not the bacterium itself are responsible for the foodborne illness. The pathogen will produce the toxins (proteins of low molecular weight; ca 30 kDa) while growing in the food, which will lead to an illness when they are ingested by a susceptible person.

Characteristics of *S. aureus*
*S. aureus* is considered to cause the most common bacterial food intoxications. *S. aureus* is a feared hospital pathogen, sometimes very virulent and often resistant to antibiotics. Only enterotoxin producing staphylococci cause food poisoning. The ability to produce enterotoxin is associated with production of coagulase and heat-resistant DNase.

*S. aureus* (0.5–1.5 μm in diameter) is a gram positive, catalase positive, ubiquitous, and nonmotile microorganism. The word "staphyle" in the Greek language means a bunch of grapes, and the word "coccus" means round. *S. aureus* has a coccus shape, and occurs in clusters of irregular arrangement like the bunch of grapes. It may occur singly, in pairs, or in short chains too. *S. aureus* produces a variety of extracellular enzymes and metabolites. The most important metabolite produced is a group of heat-stable toxins called enterotoxins (staphylococcal enterotoxins) which are the agents of staphylococcal intoxications. *S. aureus* is facultative anaerobic microorganism, however, it grows best in aerobic conditions and some oxygen must be present for the production of toxins.
S. aureus growth requirements
Given adequate nutrients, time, temperature, pH, water activity (a_w), and atmosphere, contaminating S. aureus may multiply and many strains may produce enterotoxins. The following are some of the criteria:
1. A certain amount of organic compounds (i.e. amino acids) must be available to the organism as nutrients.
2. Temperature growth range is 7–47.8°C with an optimum of 35–37°C. Enterotoxins are produced at 10–46°C with an optimum of 40–45°C.
3. A growth medium/environment that is free of salt, although the bacterium can tolerate a concentration up to 10–20% NaCl.
4. Some sucrose available in growth medium/environment, although the bacterium can grow at a sucrose concentration up to 50–60%.
5. A pH range between 4.0–9.8, with an optimum of 6.0–7.0
6. Water activity (a_w) for growth can be as low as 0.86 under aerobic conditions, and 0.90 under anaerobic conditions. The optimum is >0.99, and some strains can grow at an a_w of 0.83.

The precise growth at a certain level of a parameter is dependent on the degree to which all other parameters are at optimal levels. The interaction between these parameters is important for the growth of the microorganism. Also, it has been found that the production of the enterotoxins tends to be favored by the organism's optimum growth conditions. They appear in cultures, and increase gradually during all phases of growth.

Ecology of S. aureus growth
S. aureus do not compete with the normal flora of most foods, and this is especially true for those that contain large numbers of lactic acid bacteria where conditions permit the growth of the latter organisms (i.e. raw foods). At temperatures that favor staphylococcal growth, the normal food saprophytic flora offers protection against staphylococcal growth through organisms competition for nutrients and modification of the environment to conditions less favorable to S. aureus. However, cooking eliminates the normal competitive flora; therefore, it is in the prepared foods that the growth of S. aureus may be permitted. This requires recontamination of the food (e.g., during slicing of baked ham) since S. aureus is also killed by cooking. Examples on bacterial types known to be antagonistic to S. aureus include: Acinetobacter, Aeromonas, Bacillus, Pseudomonas, S.epidermis, the Enterobacteriaceae, Lactobacillaceae, enterococci, etc.

S. aureus enterotoxins
The staphylococci produce 12 enterotoxins. These include: A (SEA), B (SEB), C (SEC), D (SED), E (SEE), G (SEG), H (SEH), I (SEI), J (SEJ), K (SEK), L (SEL), and M (SEM). Possibly there are some unidentified ones. Three variants of SEC were described (C1, C2, C3) based on minor antigenic differences. S. aureus enterotoxins are somehow chemically and serologically related but not identical. They may differ in certain physicochemical properties, yet each has about the same potency. All are simple proteins. Enterotoxin A is the most toxic and the one most frequently associated with the poisoning outbreaks. It has been indicated that less than 1 μg of this toxin can result in illness. SED is the second most frequent toxin. There is no enterotoxin F (SEF). At one point, toxin shock syndrome toxin was misidentified as SEF. This is no longer the case.

S. aureus enterotoxins (simple proteins) are hygroscopic, easily soluble in water and salt solutions, and resistant to trypsin, chymotrypsin and papain. Pepsin destroys the toxin at pH 2.0, not at higher pH. The toxin is resistant to radiation (200 kGy) and boiling (may resist 121.1°C, 250°F, for up to 0.5 hr).

Pathogenesis (target area)
Since contaminated food products harbor the organism, it is expected that the enterotoxins will act on the receptors in the gut that transmit impulse to medullary centers.

Toxigenic dose and onset of illness
A population of a million or more S. aureus cells/gram in food is considered necessary for sufficient toxin production to arouse symptoms of poisoning. The minimum quantity or dose needed to cause illness in humans is about 200 ng. Smaller amounts have been reported present in foods — 10–50 ng/g of food. Onset of illness is less than 30 min to 8 hr following the ingestion of toxin containing food. Most illness, however, occurs within 2–4 hr, and recovery is within 24–48 hr with, no lasting effects. This illness is rarely fatal (usually in very young children or older individuals).

Clinical features (symptoms)
S. aureus enterotoxins cause severe gastroenteritis (inflammation of the intestinal tract lining), nausea, vomiting, retching, abdominal cramps, sweating, chills, prostration, weak pulse, shock, shallow respiration, subnormal body temperatures. This intoxication is rarely fatal, but the reactions are so severe that the affected persons may wish they had died. Treatment of patients consists of bed rest and maintenance of body fluids and electrolytes.
**Method of transmittance/implicated foods**

*S. aureus* is transmitted to susceptible individuals through ingestion of food contaminated with the microorganism. A variety of foods have been implicated with staphylococcal foodborne poisoning; especially proteinaceous ones. Examples include foods of animal origin (i.e. beef, pork, turkey, chicken, fish, eggs) whether cooked, chopped or comminuted; cream-filled custard pastries; some dairy products; hollandaise sauce; bread pudding; potato salad; other meat salads; and warmed-over foods.

In general, it can be said that for staphylococcal poisoning to occur four things must take place:

1. The food must be contaminated with enterotoxin-producing staphylococci.
2. The food must be capable of supporting the growth of the contaminant.
3. The food must be held at a temperature sufficiently high and for a sufficient period of time to permit sufficient growth to result in the formation of enough toxin.
4. The food must be eaten.

**Survival and growth in some foods**

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Growth</th>
<th>No growth</th>
<th>Killed</th>
</tr>
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<tbody>
<tr>
<td>Raw turkey meat</td>
<td>5 days, 15–20°C</td>
<td>5 days, 7–10°C</td>
<td></td>
</tr>
<tr>
<td>Chicken salad</td>
<td>24 hours, 22–32°C</td>
<td>24 hours, 24°C</td>
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<tr>
<td>Ham salad</td>
<td>24 hours, 32°C</td>
<td>24 hours, 4–22°C</td>
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<tr>
<td>Cooked fish mince, surimi</td>
<td>27 hours, 25°C</td>
<td>5 days, 5 ºC</td>
<td>6 weeks, 5 ºC</td>
</tr>
<tr>
<td>Tofu</td>
<td>24 hours, 5–25°C</td>
<td></td>
<td></td>
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<tr>
<td>Pumpkin pie</td>
<td>48 hours, 25–35°C</td>
<td></td>
<td>84 hours, 4 ºC</td>
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<tr>
<td>Oatmeal raisin cookies</td>
<td></td>
<td></td>
<td>24 hours, 25 ºC</td>
</tr>
<tr>
<td>Cream puff filling</td>
<td>24 hours, 25 ºC</td>
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</tbody>
</table>
How is *S. aureus* introduced to food?

Humans are primary reservoirs of *S. aureus*. It is commonly found in the nose, throat, hands, fingertips, hair and skin. It is estimated that about 30–50% of healthy individuals carry this microorganism in their nasal passages, throat, hair and skin. This means that any food that requires handling and preparation is susceptible to contamination through nasal discharges (coughing and sneezing), skin infections (acne, pimples, boils, scratches, and cuts). About 40–50% of *S. aureus* isolates from healthy individuals are enterotoxin producers. *S. aureus* is also found on the skins or hides of animals, thus cross contamination may result from these animals during slaughtering. It can also be found in the environment — air, dust, water, and waste (of human and animal origins).

Preventive or corrective procedures

The five factors most frequently found to be involved in food contamination are:

1. Preparing foods far in advance of planned service
2. Infected persons practicing poor personal hygiene.
3. Inadequate cooking or heat processing.
4. Holding food in warming devices at bacterial growth temperatures.
5. Inadequate refrigeration.

Therefore, proper handling, heat processing, cooling and refrigeration are important control measures. Perhaps the control of the temperature is the most effective means to restrict this type of poisoning. Adequate refrigeration and exclusion of unhealthy food handlers may minimize the risk too. A departure from a single operating condition will hardly result in an unsafe food product. If negligence reaches a point where all of these sanitary conditions are disregarded (or at least several of them are combined), then a hazardous outcome may result. Thus, it can be said that since the organism *S. aureus* is ubiquitous, so good sanitation of the food handling and processing environment is necessary to prevent control of its growth and toxin production. The organism can spoil a variety of foods other than being a food pathogen, and usually there is no production of off-odors in food. Therefore, the presence of these bacteria or their toxins in food would not be detectable by sensory methods. Preventive measures such as heat processing and normal cooking temperatures may be sufficient to kill the bacterial cells. However, the enterotoxins are not inactivated by this, for they are thermostable. Hence, the absence of viable staphylococci cells does not guarantee the safety of the food to be eaten, and by no means do the current commercial practices provide absolute safeguards against this.

Diagnosis and detection of the organism and enterotoxin
Diagnosis is based on clinical signs and symptoms and the nature of the suspect food.

Often large numbers of staphylococci are present in the food, but not if the food has been heated (boiled), which will destroy the cells but not the toxin. The enzyme thermonuclease (TNase), a heat-stable DNase, persists in cooked food, and has been used diagnostically for *S. aureus*.

Staphylococci can be detected by culturing in trypticase soy broth with 10% NaCl followed by plating on mannitol salt agar or Baird-Parker agar; plating can also be done directly.

Enterotoxin can be detected by immunodiffusion (slide test), reverse passive latex agglutination assay or by ELISA. Minimum acceptable detection level is 0.5 ng/g; kits available that can detect 0.2.

**Selected References**


**Note:** Much of the present information about staphylococcal food poisoning was provided through research conducted at the Food Research Institute, University of Wisconsin, especially by Dr. M. Bergdoll and his associates.