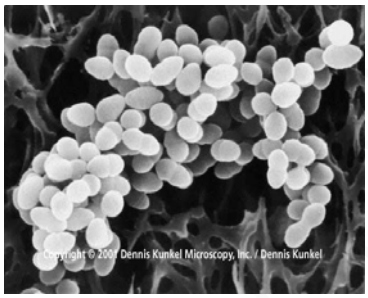
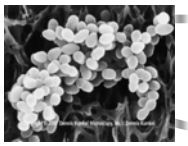
Staphylococcus aureus



Introduction

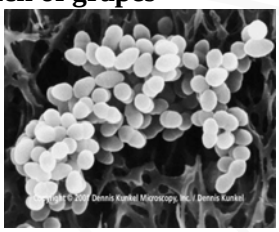
- In 1878, Koch observed staphylococci.



- *Staphylococcus* recognized as a separate genus in 1880 by Pasteur.

S. aureus Properties

- **In the Greek language:**
  - **staphyle = a bunch of grapes**
  - **coccus = round**



Introduction

- 1884 - Rosenback grew staphylococci on a solid medium.
- 1884 - Sternberg associated staphylococci with “ptomaine” formation in cheese that caused human illness.

Introduction

- 1894 - Denys associated illness with eating 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.

Introduction

- 1914 – Barber related staphylococcal food poisoning to a toxic substance produced in food.
- He isolated staphylococci from contaminated milk that came from a sick cow with mastitis.

Introduction

- 1929 - Dack studied an outbreak of food poisoning caused from eating X-mas cake.
- Re-discovered the role of staphylococci in food poisoning.
- He showed with human volunteers that the isolated staphylococci produced a toxic substance in culture, this substance caused typical staphylococcal food poisoning.

Introduction

- 1948–1974 studies demonstrated:
  - ➢The presence of preformed enterotoxin in foods that had caused staphylococcal food poisoning.
  - ➢Antitoxin in the blood of people that had suffered from this type of poisoning.

S. aureus in the US (estimated)

Agent	Cases	%	Deaths	%
S. aureus	185,060	1.3	2	0.1
Total bacterial	4,175,565	30.2	1,297	71.7
Total foodborne	13,814,924	100	1,809	100

S. aureus

- In 1994, *S. aureus* was considered to be the cause of one of the most common bacterial food intoxications.
- Holt et al. (1994) estimated *S. aureus* food intoxication to be the second most prevalent disease in the US.

Contemporary Problems

- Foods associated with staphylococcal food poisoning:
  - ➢In the US
    - – Meat products (e.g., ham)
    - – Desserts
  - ➢In Japan – Rice balls
- Seasonal variations

Illness & Causative Agent

- *S. aureus* causes foodborne intoxication.
- The thermostable enterotoxins and not the bacterium are responsible for the foodborne illness.
- Staphylococcus / staphylococcal food poisoning.

Illness & Causative Agent

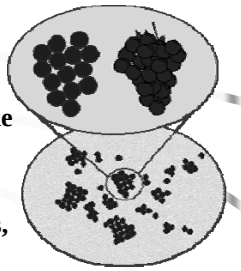
- The pathogen produces the toxins while growing in the food.
- When the toxins are ingested by a susceptible person they will cause the illness.

S. aureus Properties

- Only enterotoxin-producing staphylococci cause food poisoning.
- The ability to produce enterotoxin(s) is associated with production of coagulase and heat resistant DNase.

S. aureus Properties

- **It has a coccus shape.**
- **Occurs in clusters of irregular arrangement like the bunch of grapes.**
- **May occur singly, in pairs, or in short chains.**

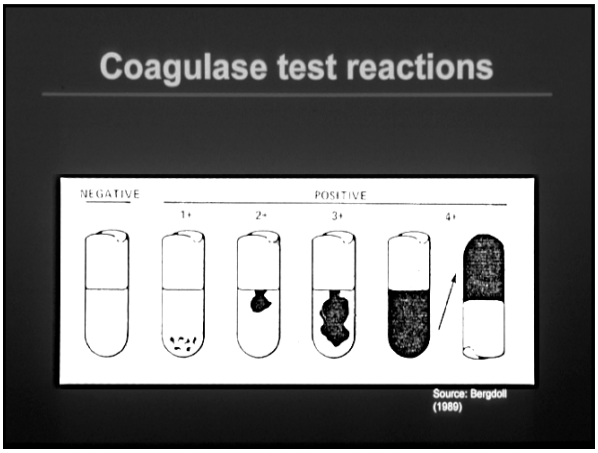


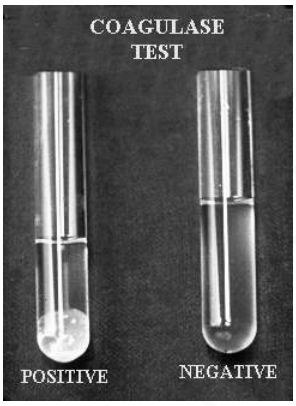
S. aureus Properties

- ***S. aureus* is ~0.5-1.5 µm in diameter**
- **Gram positive, non-sporeforming, non-motile, facultative anaerobe**
- **Coagulase and catalase positive**

Coagulase Test

- Suspect colonies are incubated in 2 ml of Brain Heart Infusion (BHI) broth for 18–24 hr at 35–37°C.
- 0.5 ml coagulase plasma (with 0.5 ml of EDTA) is added to 0.5 ml of broth culture and mixed.
- Tubes are incubated and examined after 4 hr.





S. aureus Properties

- ***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).**

S. aureus Properties

- **Temperature range: 7–47.8°C (Opt. 35–37°C)**
- **Enterotoxins produced between 10–46°C (Opt. 40–45°C)**

S. aureus Properties

- pH range: 4.0 – 9.8 (Optimal 6-7)
- Salt tolerant (10 – 20% NaCl)

S. aureus Properties

- Can grow at a sucrose concentration up to 50–60%
- Water activity as low as 0.86 under aerobic conditions, and 0.90 under anaerobic conditions.
- Greater toxin production under aerobic conditions.

Environmental Effects

- ≥10% NaCl inhibits SEA and SEB production.
- Enterotoxins are not formed:
  - Below pH 5.3 at 30°C
  - Below pH 5.6 at 10°C
- Minimal water activity -- 0.86 for growth

Water Activity

- Enterotoxin production occurs at 0.86 – 0.99, Opt. 0.99
- Reducing a_w minimizes production of enterotoxins:
  - 0.90 a_w reduces SEB by 90-99%

(Maradona, 1998)

Microbial Ecology

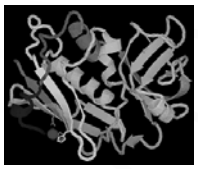
- *S. aureus* does not compete well with the normal flora of most foods.

S. aureus Toxins

- *S. aureus* is the common species associated with food intoxication.
- 12 enterotoxins: A, B, C, D, E, G, H, I, J, K, L, M.
- Three variants of SEC – C_1, C_2, C_3 (minor antigenic differences)

S. aureus Toxins

- Staphylococcal enterotoxin A (SEA) most common in gastroenteritis.



S. aureus Toxins

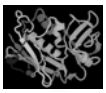
- Enterotoxins are simple proteins.
- Easily soluble in water and salt solutions.
- Resistant to trypsin, chymotrypsin, and papain.
- Pepsin destroys the toxin at pH 2.
- Toxin is resistant to radiation (200 kGy), and boiling (resists 121.1°C for 0.5 hr)

S. aureus Enterotoxins

- Low molecular weight (~30 kDa) simple proteins
- Heat resistant simple
- *S. aureus* itself is not heat resistant.
- Enterotoxins A and D are the most heat resistant.
- When active, A and D exhibit proteolytic enzyme resistance.

Pathogenesis – target area

- Enterotoxins expected to act on the receptors in the gut that transmit impulse to medullary centers.



Clinical Symptoms

- ***S. aureus*** **enterotoxins cause:**
  - **severe gastroenteritis**
  - **nausea, vomiting, retching, abdominal cramps, sweating, chills, prostration, weak pulse, shock, shallow respiration, subnormal body temperatures.**

S. aureus Food Poisoning

- **About ≥10^6 cells/gram of *S. aureus* in food is needed for toxin production.**
- **About 200 ng of toxin can cause illness in humans.**

S. aureus Food Poisoning

- **Onset of illness takes <30 min – 8 hr. following ingestion of the toxin containing food.**
- **Most illness, however, occurs within 2–4 hr.**
- **Recovery is within 24–48 hr.**
- **Illness is rarely fatal.**

Clinical Symptoms

- **The enterotoxins acts on the receptors in the gut that transmit impulse to medullary centers.**
- **Treatment of patients consists of bed rest and maintenance of body fluids and electrolytes.**

S. aureus Infections

- ***S. aureus*** **is a feared hospital pathogen.**

- **Sometimes it can be very virulent, and often resistant to antibiotics.**

How Is *S. aureus* Introduced to Food?

- ***S. aureus* is commonly found in:**
  - **Nose**
  - **Throat**
  - **Hands**
  - **Fingertips**
  - **Hair and skin**
- **Found in more than 50% of healthy people.**
- **Found on skins or hides of animals.**
- **Found in the environment.**

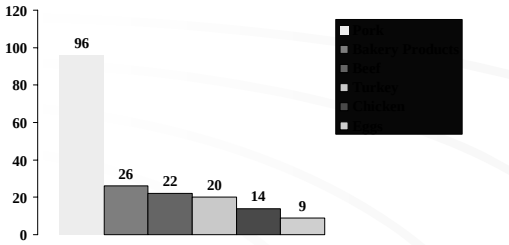
How Is *S. aureus* Introduced to Food?

- **Any food that requires handling and preparation is susceptible for contamination.**
- ***S. aureus* is also found on the skin or hides of animals.**
- **Cross-contamination may result from these animals during slaughtering.**

Foods Often Incriminated

- **Meats and meat products**
- **Poultry and Fish**
- **Cream-filled baked goods**
- **Baked foods**
- **Potato Salad**
- **Salads containing any of the above items**
- **Any nutrient-rich, moist food that is temperature abused.**

Food Sources for Staphylococcal Outbreaks (1973-1987)



(Bean et al., 1990)

Contributing Factors

- **Improper storage and holding temperatures**
- **Inadequate cooking/processing temperatures**
- **Contaminated Equipment**
- **Unsafe food sources**
- **Poor personal hygiene**
  - **10 – 50% adults are reservoirs of *S. aureus***

Prevention

- **Adequate storage and refrigeration of foods**
- **Not preparing foods far in advance**
- **Adequate cooking and/or heat processing**
- **Avoiding poor personal hygiene**
- **Not holding foods between 40 – 140°F (4.4–60°C) for prolonged periods**
  - **40-135°F (4.4-57°C); new numbers**

Pathogen Detection

- Laboratory media:
  - Trypticase soy broth with 10% NaCl
  - Mannitol salt agar
  - Baird-Parker agar

Indicators for the Presence of *S. aureus*

- **Coagulase Test**
- **Thermostable Nuclease Test (TNase)**
- **Polymerase Chain Reaction (PCR)**

TNase Testing

- **Culture is boiled for 15 min.**
- **Toluidine blue agar plates are prepared.**
- **2 mm wells are dug in the plates and filled with the boiled cultures**
- **Plates incubated for 2–4 hr at 37–50°C**
- **Pink halos around wells indicates positive reaction.**

(Maradona, 1998)

Polymerase Chain Reaction (PCR)

- **Thermostable DNA polymerase catalyzes the gene probe amplification.**
- **Amplified DNA is detected by hybridization ring using radio- and non-radiolabeled probes.**
- **Can amplify a single DNA molecule to 10^7 molecules.** **(Maradona, 1998)**

Detection Methods of Enterotoxins

- **Biological**
- **Immunological (many, including kits)**

Biological Detection

- **Each new toxin type had to be detected biologically**
- **Biological subjects used are cats, kittens, and monkeys.**
- **Kittens--emetic response**
- **Can determine the enterotoxin activity by observing responses.**
- **Monkeys used to simulate human response.**

(Maradona, 1998)

Immunological Detection

- Microslide
- Agglutination
- Radioimmunoassay (RIA)
- Enzyme Linked Immunosorbent assay (ELISA)
- Enzyme Linked Fluorescent Inmmunoassay (ELFA)

Microslide Test

- **Linear migration of antibody and antigen in a gel**
- **AOAC recommended method**
- **Sensitivity level of 50 ng/ml**
- **Easy to read results**
- **Disadvantages**
  - **Must concentrate sample from 100 g to 0.2 ml**
  - **Time consuming (1-3 days)**

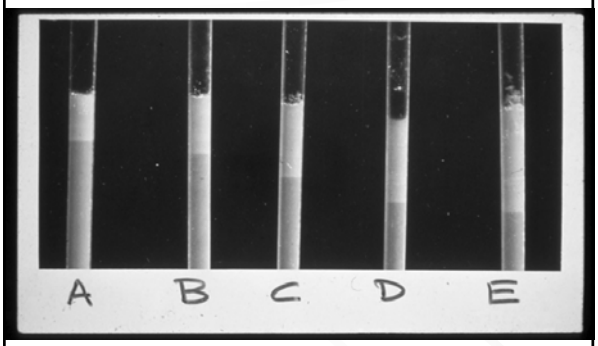
(Maradona, 1998)

Gel Diffusion

- **Agar is prepared with antiserum and aspirated into Pasteur pipette.**
- **Pasteur pipette is sealed.**
- **Liquid sample is added on top of solidified agar.**
- **Pipettes are incubated at 37°C for 24 hr.**
- **Precipitant band is formed if toxin is present.**

(Fung, 1998)

Gel Diffusion Bands of *S. aureus* Enterotoxins



Source: Dr. D.Y.C. Fung

Radioimmunoassay (RIA)

- First sensitive test for enterotoxin (<1ng/ml)
- Reliable at 10 ng
- 5–20 times more sensitive than Microslide
- Radioactive tracer labels (^{125}I) for enterotoxin antibody reaction.

(Maradona, 1998)

RIA

- Results in 3–4 hr
- Disadvantages
  - Radioactive material
  - Purified enterotoxin required

(Maradona, 1998)

Enzyme Linked Immunosorbent Assay (ELISA)

- Enzyme reacts with substrate causing a visible color change.
- Color change is dependent on SET concentration.
- Sensitivity 0.2 – 0.7 ng/ml
- Results in ~ 3 hr
- Commercially available RIDASCREEN by R-Biopharm

Source: Bio-Tek

Enzyme Linked Fluorescent Immunoassay (ELFA)

- Enzyme converts substrate into fluorescent product.
- Optical scanner reads intensity which is proportional to enterotoxin present
- Sensitivity of 0.1 – 0.8 ng/ml
- Commercially available from bioMerieux Vitek: Vidas SET

Source: bioMerieux Vitek