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.

\[ S. aureus \]

\begin{tabular}{|l|c|c|c|}
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Agent & Cases & % & Deaths & % \\
\hline
\textit{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 \\
\hline
\end{tabular}

\[ S. aureus \]

• In 1994, \textit{S. aureus} was considered to be the cause of one of the most common bacterial food intoxications.

• Holt et al. (1994) estimated \textit{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

• \textit{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**

- **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)

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**Microbial Ecology**

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

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**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 – C₁, C₂, C₃ (minor antigenic differences)

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**S. aureus Toxins**

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

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**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)

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**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 $\geq 10^6$ cells/gram of *S. aureus* in food is needed for toxin production.

- About 200 ng of toxin can cause illness in humans.

Clinical Symptoms

- The enterotoxins act 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* 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.

*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.

- 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)

![Bar chart showing food sources for staphylococcal outbreaks](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 Immunoassay (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 (¹²⁵I) 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