STAPHYLOCOCCI

• Reading Assignment: Murray et al., Medical Microbiology, 8th ed., 2016. Chapter 18

STAPHYLOCOCCI

OBJECTIVES

• Describe the epidemiology of staphylococcal infection.
• List severe disease syndromes in which Staphylococcus aureus should be considered in the differential diagnosis
• List virulence factors associated with S. aureus
• List the clinically significant species of coagulase-negative staphylococci and describe the diseases they are associated with
• List lab tests that help differentiate Staphylococcus species
BEFORE WE BEGIN.....

How do we study microbiology?
- Organism
- Specimen Type
- Clinical Presentation
- Other

The Gram Stain

Gram Positive

Gram Negative
I'm *Staph aureus*; I dish out disease.
I can make you quite ill if I please.
I’m golden: woe betide you
If I get inside you.
You’ll wish that you only had fleas.

Adapted from a poem by Celia Warren on OEDILF

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Catalase Test

- Enzyme that breaks hydrogen peroxide (H₂O₂) into H₂O and O₂.
- Bubbling that is seen is due to the evolution of O₂ gas.
- H₂O₂ is a potent oxidizing agent that damages cells; any cell that uses O₂ or can live in the presence of O₂ must have a way to get rid of the peroxide.
Catalase Test
Differentiate Staph from Strep

Staphylococci are **catalase +**
Streptococci/Enterococci are **catalase -**

Micrococcus and Staphylococcus
- Commonly recovered from environment or as commensals inhabiting skin and mucus membranes
- Certain staphylococci are pathogenic for humans
- Micrococcus are not typically associated with disease and not considered clinically significant when isolated from human specimens
MHD Staphylococci

Staphylococci
Important Species

• S. aureus
• S. auricularis
• S. capitis
• S. cohnii
• S. epidermidis
• S. hemolyticus
• S. hominis
• S. lugdunensis
• S. saccharolyticus
• S. saprophyticus
• S. simulans
• S. warneri
• S. xylosis

CASES

• Clinical presentations and risk factors associated with organism
• Highlight virulence factors

Case 1

• A 26 year old marine recruit presents to the base medic
• Exam reveals large, pus-filled lesions surrounded by erythema on both legs

Case 1 Diagnosis

Cellulitis/abscess of lower leg caused by *Staphylococcus aureus*

- Pyogenic (makes pus)
- Pain, edema, erythema, accumulation of purulent material
- High inoculum required in immunocompetent host
- Often following trauma or introduction of foreign body

*Staphylococcus aureus*

Key Characteristics

- Gram positive cocci in clusters
- Golden beta-hemolytic colonies
- Catalse +

Habitat

- Found in external environment
- Found on skin and mucous membranes
  - Anterior nares - 20-40% of adults
  - Intertriginous skin folds
  - Perineum
  - Axillae
  - Vagina
- Significant opportunistic pathogen under appropriate conditions
**Staphylococcus aureus**

*Serious Infection*

- Usual sites of infection are those in which the organism is part of normal flora
  - **Skin**: folliculitis, impetigo, furuncles, carbuncles; postsurgical wound infections
  - **Nose and throat**: sinusitis, peritonsillar abscesses; mastoiditis, bronchitis and staphylococcal pneumonia
  - **GI tract, urethra, vagina**: enterocolitis, cystitis, prostatitis, cervicitis, salpingitis, pelvic abscess

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**Staphylococcus aureus**

*Serious Infection – Furuncle*

- **Furuncle** (or boil) skin disease caused by infection of hair follicles, resulting in localized accumulation of pus and dead tissue
- Red, pus-filled lumps that are tender, warm, and extremely painful.
- A yellow or white point at center of lump can be seen when boil is ready to drain

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**Staphylococcus aureus**

*Serious Infection – Carbuncle*

- A carbuncle is an abscess larger than a boil, usually with one or more openings draining pus onto the skin.
- Carbuncles may develop anywhere, but they are most common on the back and the nape of the neck.
What’s in the Bug Tool Box?
• Adhere
• Evade
• Destroy

What’s in the Bug Tool Box?
• Adhere = Cell surface
• Evade = Protein A
• Destroy = Cytotoxins
  Enzymes

Staphylococcus aureus
Virulence Factors
Components that interfere with phagocytosis (Evade)
  – Protein A - interfering with opsonization and ingestion of organism by PMNs
**Staphylococcus aureus**  
**Virulence Factors**

**Cytotoxins (Destroy)**
- Hemolysins—The “Lysin” Family  
  Lyse RBCs, leukocytes
  - α-hemolysin: Pore former **Tissue damage**
  - β-hemolysin: Sphingomyelinase
  - δ-hemolysin: Surfactant
  - γ-hemolysin: Pore former

**Enzymes (Destroy)—The “A ses”**
- **Coagulase**
  - conversion of fibrinogen to fibrin
**Staphylococcus aureus**

**Virulence Factors**

Enzymes (Destroy)

— cell to cell spread

- Fibrinolysin - break down fibrin clots
- Hyaluronidase - hydrolyzes intercellular matrix connective tissue
- Lipase - survival in sebaceous material

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**Case 2**

- 18 year old girl presents to ED 3 weeks after a knee injury while playing baseball
- Right now knee “feels like jelly” and is extremely painful
- History of ‘spider bite cellulitis’ on shin 1 year ago

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**Case 2 Diagnosis**

Septic Arthritis and Osteomyelitis of Knee

- Hematogenous dissemination or secondary infection
- Pain and fever
- Blood culture + in 50%
- Replaced by *Neisseria gonorrhoeae* in sexually active persons
Clinical Considerations

- Differential diagnosis of skin and soft tissue infections (SSTI’s) compatible with *S. aureus* infection:
  - Abscesses, pustular lesions, “boils”
  - “Spider bites”
  - Cellulitis

Clinical Considerations

- Differential diagnosis of severe disease compatible with *S. aureus* infection:
  - Sepsis syndrome
  - Osteomyelitis
  - Necrotizing pneumonia
  - Septic arthritis
  - Necrotizing fasciitis

**Staphylococcus aureus**

Factors Predisposing to Serious Infection

- Defects in leukocyte chemotaxis
  - Congenital (Wiskott-Aldrich syndrome, Down’s syndrome, Job’s syndrome, Chediak-Higashi syndrome)
  - Acquired (Diabetes mellitus, rheumatoid arthritis)

- Defects in opsonization by antibodies secondary to congenital or acquired hypogammaglobulinemias or complement component
Staphylococcus aureus
Factors Predisposing to Serious Infection

• Defects in intracellular killing of bacteria following phagocytosis due to inability to activate the membrane bound oxidase system (CGD, lymphoblastic leukemia, acute and chronic myelogenous leukemia)
• Skin injuries (burns, surgical incisions, eczema, sports injuries)
• Presence of foreign bodies (sutures, IV lines, prosthetic devices)

Case 3

• A 3 month old baby presents to her pediatrician
• Over the past 3 days her mother has seen erythema spread from around her mouth to cover her entire body
• Slight pressure displaces the skin and large cutaneous blister (bullae) form soon after, followed by sloughing off of the skin layer (desquamation of the epithelium)
• The blisters contain clear fluid

Case 3 Diagnosis

Staphylococcal scalded skin syndrome
• Neonates and young children
• Epithelium heals when antibodies are present 7-10 days later
• No bacteria or leukocytes are present in fluid; organisms around eyes, nose, mouth
**Staphylococcus aureus**

**Virulence Factors**

Toxins (Destroy)

- **Exfoliatins or Exfoliative Toxins**
  - Dissolves the mucopolysaccharide matrix of epidermis
  - Causing separation of skin layers

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**Case 4**

- 18 people attending a retirement party became ill approximately 3-4 hours after eating
- Symptoms included nausea, vomiting, and diarrhea
- Relatively few people had headache or fever
- Symptoms lasted 24 hours
- Symptoms were associated with eating ham

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**Case 4 Diagnosis**

Staphylococcal Food Poisoning

- Severe vomiting, nausea, diarrhea, cramping, headache, but not fever
- Intoxication ≠ Infection
- 4 hour incubation period
- Processed meats, custard-filled pastries, potato salad, ice cream
- Contamination = colonization
**Staphylococcus aureus**

**Virulence Factors**

**Toxins (Destroy)**

- **Enterotoxins**
  - Toxin is **heat-stable**
  - “Re-heating” kills bacteria but not toxin
  - Not produced by ingested Staphylococcus

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**Case 5**

- A 15 year old girl was admitted to the hospital with a 2 day history of pharyngitis and vaginitis
- Vomiting and watery diarrhea
- Febrile and hypotensive (low blood pressure) on admission
- Diffuse erythematous rash over entire body
- Disseminated intravascular coagulation (DIC) and severe thrombocytopenia
- Chest radiograph showed bilateral infiltrates

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**Case 5 Diagnosis**

Staphylococcal Toxic Shock Syndrome

- Multi-organ toxicity
- Fever, hypotension, rash
- Entire skin desquamates
- Fewer than 100 cases in US each year
- 5% fatality unless not treated with antibiotics
Staphylococcus aureus

Virulence Factors

Toxin (Destroy)
- Localized growth followed by release of toxin
- **Superantigens**
  - Toxic shock syndrome toxin-1 (TSST-1)
  - Non-specific activation of T-cells (MHC class II)
  - Polyclonal T-cell proliferation
  - Massive cytokine release

Case 6

- A 21 year old female presents to an urgent care clinic
- Her symptoms include burning and pain during urination (dysuria)
- She is sexually active in a new relationship

Symptoms of Urinary Tract Infection

- Frequent urination
- Burning and pain during urination
- Cloudy urine
- Fever and chills
- Nausea and vomiting
- Blood in the urine
- Inability to urinate

- Bladder discomfort
- Abdominal pain
- Fatigue
- Nighttime frequency

- Lower back pain
- Sensitivity to touch

- Increased frequency
- Urgency
- Incontinence
Case 6 Diagnosis

UTI caused by *Staphylococcus saprophyticus*
- Cause of acute urinary tract infection in young women
- 2nd most common cause of uncomplicated cystitis (after *E. coli*) among women of college and child-bearing age
- Identification based on negative coagulase and resistance to novobiocin

Case 7

- 36 year old cocaine user presents to the emergency department
- Acute onset weakness of right extremity
- 10 weeks of fever, chills, malaise, shortness of breath
- On admission she had tachycardia, hypotension, fever, pansystolic murmur
- Multiple positive blood cultures

Case 7 Diagnosis

Native Valve Endocarditis caused by *Staphylococcus lugdunensis*
- Persistent bacteremia
- Often requires valve replacement
- May be associated with secondary complications such as brain infarct caused by septic emboli
**Staphylococcus lugdunensis**

- Colonizes human inguinal area
- More virulent than other ‘coag-negative Staph’
- Native-valve, prosthetic-valve, and pacemaker-associated endocarditis
- Staph species most commonly associated with native valve infection
- Causes variety of human infections including skin and soft tissue abscesses, device-related infection

**Staphylococcus epidermidis**

- Most frequently isolated clinically significant coagulase-negative staphylococci
- Associated with infections of indwelling devices
- Virulence related to production of extracellular slime that promotes adherence of organism to surfaces of foreign bodies forming biofilm
- Biofilm also protects organisms from antimicrobial agents, therefore removal of foreign bodies often necessary for resolution of infection

**Lab Tools**

- Gram stain
- Surface Structures
- Protein A
- The A ses
- The Lysins
**Staphylococcus aureus**

**Laboratory Identification**

- **Coagulase #1**
  - Cell-free or ‘unbound’
  - Binds to prothrombin catalyzing conversion of fibrinogen to fibrin
  - “Tube coag”
  - Rare *S. aureus* may be coagulase-negative and some animal isolates (*S. intermedius, S. hyicus, S. delphini, S. schleiferi subsp. coagulans*) may be tube coagulase-positive

- **Coagulase #2**
  - Cell-free or ‘unbound’ aka: clumping factor
  - Directly converts fibrinogen to fibrin
  - “Slide coag”
  - Latex agglutination assay detecting **protein A** and **coagulase** (clumping factor), monoclonal antibodies

Showing positive (left circle) and negative (right circle) agglutination for the StaphTEX™ Blue Kit
Methicillin Resistant *Staphylococcus aureus* (MRSA)

- The “Chief of Staph”
- Responsible for hospital-associated and community-associated Staph infection
- Resistant to all beta-lactam antibiotics

MRSA – *mecA* Gene

- *mecA* is carried on a mobile genetic element called "staphylococcal cassette chromosome mec" (SCCmec)
- Encodes for altered “penicillin-binding protein 2a” (PBP2a)
- Has decreased binding affinity for beta-lactam antibiotics and allows peptidoglycan synthesis even in the presence of beta-lactam antibiotics

Who Gets Staph or MRSA Infections?

- Staph infections, including MRSA, occur most frequently among patients in hospitals and healthcare facilities (such as nursing homes and dialysis centers) who have weakened immune systems
- Healthcare-associated MRSA (HA-MRSA) infections include
  - Surgical wound infections
  - Urinary tract infections
  - Bloodstream infections
  - Pneumonia
  - Central venous catheter line infections
MRSA Transmission in Hospitals

- In hospitals, the most important reservoirs of MRSA are colonized or infected patients
- Hospital personnel can serve as a link for transmission between colonized or infected patients

Both infected and colonized patients contaminate hospital environment with same relative frequency

MRSA recovery from bedrails

What can be done to control MRSA?

- Careful, compulsive hand hygiene for all patient interactions (behavioral change)
- Standard and transmission based Contact/Droplet precautions:
  - Gowns  Gloves  Masks
- Effective cleaning of the patient care environment
- Clean shared/dedicated equipment
  - Stethoscopes  BP cuffs
  - Thermometer  TV Remotes
- Appropriate use of antibiotics

Screening for MRSA

CA-MRSA Outbreaks

- Often first detected as clusters of absceses or "spider bites"
- Various settings
  - Sports participants: football, wrestlers, fencers
  - Correctional facilities: prisons, jails
  - Military recruits
  - Daycare and other institutional centers
  - Newborn nurseries and other healthcare settings
  - Men who have sex with men

An organism identified as gram-positive cocci in clusters is isolated from the urine of a 21 year female with symptoms of acute cystitis. The laboratory reports the organism is coagulase-negative and novobiocin resistant. What is the most likely identification of this bacterium?

A Staphylococcus aureus
B Staphylococcus epidermidis
C Staphylococcus lugdunensis
D Staphylococcus pyogenes
E Staphylococcus saprophyticus
What is the most important reservoir of methicillin resistant Staphylococcus aureus (MRSA) in hospitals?

A Colonized or infected patients  
B Colonized or infected medical staff  
C Clothing worn by patient care personnel  
D Child visitors who attend day care centers  
E Medical equipment

Which virulence factor is also used by the laboratory to identify Staphylococcus aureus?

A Exfoliative Toxin  
B Toxic Shock Syndrome Toxin  
C Coagulase  
D Fibrinolysin  
E Lipase