Staphylococcus

Student Lab Day 3
Division of Laboratory Sciences
Michele Jurgensmeier MT(ASCP) SM

Gram Positive Cocci

General Information
- Cause human infections
- Recover from various clinical specimens
- Found in many places
- Spread by direct contact
  - Skin and mucous membranes penetration
- Elaborate an inflammatory response
- Produce pathogenic effects

Staphylococcus species

General information
- Normal flora of skin and mucous membranes
- Most are facultative anaerobes
- Growth on nutrient media containing peptone
- Inhibited by crystal violet or high concentrations of bile salts
- Fast growing
- Non-motile and non-spore forming

Identification of Staphylococcus and other related species

Colony morphology
- Important role in clinical laboratory
- Trained microbiologist can differentiate pathogens from non-pathogens
- Economize lab testing

Staphylococcus species

General information
- Common human pathogens:
  - Staphylococcus aureus
  - Staphylococcus epidermidis
  - Staphylococcus saprophyticus
  - Staphylococcus lugdunensis

Staphylococcus species

Growth Characteristics
- Colony morphology
  - Opaque, smooth, circular
  - Gray-white, white, cream, yellow (golden)
  - Hemolysis
    - S. aureus beta-hemolytic
**Staphylococcus** and other related species

- Growth characteristics
  - Growth on sheep blood and chocolate agar
  - No growth on MacConkey agar

**Catalase test**
- Clean glass slide
- Colony on slide
- 3% H2O2 placed on colony
- Positive test shows rapid production of bubbles
- Negative test will show no production of bubbles
- Commonly used to differentiate Gram + bacteria

**Staphylococcus species**

**Identification methods**
- Slide coagulase
- Tube coagulase
- Latex agglutination
- Automated and rapid multi-test systems
- Novobiocin
- Dnase
- Molecular methods

**Slide Coagulase**
- Detects clumping factor
- Directly coverts fibrinogen to fibrin
- Organism mixed with plasma
- Positive test shows agglutination
  - Indicates *Staphylococcus aureus*
- Negative results confirmed
**Staphylococcus species**

**Tube Coagulase**
- Detects staphylocoagulase which reacts with CRF, a thrombin-like molecule
- Indirectly converts fibrinogen to fibrin
- Organism suspended in plasma and incubated
- Read at 4 hrs
- Positive clot formation
  - Indicates *Staph aureus*
- Negative results confirmed

**Staphylococcus species**

**Latex agglutination**
- Various rapid kits available
- Utilize plasma-coated carrier particle
  - Usually latex
- Plasma detects clumping factor
- IgG detects Protein A
- High specificity/sensitivity
- Commonly used in clinical laboratories

**Staphylococcus species**

**Novobiocin susceptibility**
- Coagulase negative staph identification
  - Performed on urine isolates
  - Multi-test systems
- Lawn of growth prepared
- Disk place on inoculum and incubated
- Zone sizes measured

**Staphylococcus species**

**PYR hydrolysis**
- Hydrolysis of L-pyrrolidonyl-alpha-naphthylamide with formation of the free B-naphthlamide, which combines with DMAC to form a bright red color
  - *Staphylococcus aureus* is PYR negative
  - *Staphylococcus lugdunensis* is PYR positive
  - Other species will vary in their reaction

**Staphylococcus species**

**Rapid Ornithine decarboxylase**
- Modified conventional moeller ornithine can be read at 2 to 4 hours
  - Positive reaction – development of a dark purple to violet color
  - Negative reaction – no development of color (remains pale yellow to light grayish purple or development of yellow color)
  - *Staphylococcus lugdunensis* is positive for rapid ornithine
  - Other Staphylococcal species are all negative except some rare strains of *Staphylococcus epidermidis*

**Staphylococcus species**

**Polymyxin susceptibility**
- Used to identify *Staphylococcus lugdunensis*
- Determines resistance to polymyxin B
- Useful to identify clinically significant *Staphylococcus* species
- Lawn of growth prepared
- Disk place on inoculum and incubated
- Zone sizes measured
  - Resistant (< 10 mm)
  - Sensitive (≥ 10 mm)
**Staphylococcus species**

**Other identification tests**

- Automated and rapid multi-test systems
  - Vary in accuracy for identification
  - Most systems identify the common pathogens accurately
    - *S. aureus*
    - *S. epidermidis*
    - *S. saprophyticus*

**Staphylococcus aureus**

**Confirmatory tests**

- DNase
  - Used in conjunction with other tests
- PYR tests
- Automated or rapid multi-test systems
- Molecular methods

**Staphylococcus and other related species**

- Tests to differentiate the Genus Micrococcus from the Genus Staphylococcus
  - Modified oxidase (Microdase)
  - Glucose fermentation
  - Bacitracin susceptibility

**Modified Oxidase Test**

- Tetramethyl-p-phenylenediamine dihydrochloride in dimethyl sulfoxide (DMSO)
- Blue-purple color with 30 seconds is positive test

**Glucose utilization test**

- Glucose/dextrose reagent with pH indicator
- Prolonged incubation
- Left to right: glucose non-utilizers, glucose oxidizer, and glucose fermenters

**Bacitracin susceptibility**

- Bacitracin Resistant
  - Growth up to the edge of the disk
- Bacitracin Sensitive
  - Zone size ≥ 10 mm around disk

Photos courtesy of WEB CLS
**Staphylococcus aureus**

**General information**
- Most clinically significant *Staphylococcus* species
- Recovered from almost any clinical specimen
- Mild and life threatening disease
- Nosocomial infections
- Colony morphology important diagnostic tool

**Colony morphology**
- Opaque, smooth
- Raised colony with smooth border
- White-gold (cream) in color
- Beta hemolytic

**Biochemical Characteristics**
- Catalase +
- Coagulase +
- Mannitol +
- DNase +
- Glucose fermenter
- Modified oxidase -
- Bacitracin resistant

**Mechanisms of Pathogenicity**
- Capsule
- Clumping factor
- Enzymes
  - Catalase
  - Coagulate
  - Fibrinolysin
  - Hyaluronidase
  - Beta-lactamase
  - DNase
- Toxins
  - Hemolysin
  - Exfoliative
  - Panton-Valentine Leukocidin
  - Enterotoxins
  - TSST-1
- Protein A

**Clinical Significance**
- Normal flora of various skin and mucous membranes
- Invasive organism
- Infections are suppurative
- Mild and life threatening infections
**Staphylococcus aureus**

**Clinical Significance**
- Surface and skin infections
  - Folliculitis
  - Boils (furuncles)
  - Carbuncles
  - Impetigo

**Clinical Significance**
- Toxin Mediated Disease
  - Scalded Skin Syndrome
  - Toxic Shock Syndrome
  - Food poisoning

**Clinical Significance**
- Other infections
  - Pneumonia
  - Pseudomembraneous enterocolitis
  - Wound infections
  - Endocarditis/myocarditis
  - Bacteremia/septicemia
  - Osteomyelitis
  - Septic arthritis
  - Nosocomial infections

**Antibiotic Therapy**
- Susceptibility routinely performed
  - Concerned with resistance
- Organism can produce penicillinase (beta-lactamase)
- Antibiotics sensitive to penicillinase enzyme
  - Methicillin
  - Oxacillin (used to test for that class of drugs)
  - Nafcillin

**Methicillin Resistant S. aureus**
- Resistance encoded by mecA gene
  - Alters penicillin binding proteins
- **Routine susceptibility testing will not detect resistance**
**Staphylococcus aureus**

**Methicillin Resistant S. aureus**

- Heteroresistant "S" & "R" strains coexist
  - mecA gene
- "R" strains grow more slowly
- Growth requirements:
  - Media with neutral pH
  - Cooler temperature (30-35° C)
  - 2-4% NaCl

**Methicillin Resistant S. aureus**

- Modification of routine susceptibility testing
  - Inoculum from direct method
  - Incubated at cooler temperatures
  - Media with neutral pH
  - Test held for 24 hours
  - Supplement media with 2% NaCl

**Staphylococcus aureus**

**Other methods to detect MRSA**

- Detect mecA gene and new PBP2a
  - Latex agglutination
    - Detects PBP2a penicillin binding protein
    - Confirms MRSA
  - Nucleic acid probes or PCR amplification
    - Detects mecA gene
    - Gold standard for MRSA detection
  - Cefoxitin disk
    - Induces greater expression of mecA gene for detection of methicillin resistance

**Mannitol Salt Agar**

- Selective and differential primary culture media
- Used to isolate from mixed flora
- High concentration of salts inhibit other organisms
- Mannitol is sole carbohydrate

**CHROMagar/Spectra agar**

- Selective and differential primary culture media
- Incorporates chromogenic substrates
- Selective agents to inhibit other organisms
- CHROMagar incorporates cefoxitin in the media

**Oxacillin Screen Agar**

- Mueller-Hinton agar with 4% NaCl and 6 ug Oxacillin
- Used to screen for Methicillin (Oxacillin) resistance
- Useful in screening patients/health care workers for carriers
Coagulase Negative *Staphylococcus*

### Clinical Significance
- Normal flora of skin and mucous membranes
- Increasingly associated with infection
  - Prosthetic devices
  - Intravascular catheters
  - Prolonged surgical procedures
  - Immunosuppressed/immunocompromised
- Primarily hospital acquired
- *Staph. epidermidis* is the most frequently isolated coagulase negative staph

### Colony morphology
- Opaque
- Smooth, raised
- Gray- white in color
- Non-hemolytic

**Staphylococcus epidermidis**

### Biochemical Characteristics
- Coagulase -
- Mannitol -
- DNase –
- Novobiocin “S”

### Mechanisms of Pathogenicity
- Capsule
- Extracellular slime substance
  - Inhibit immune functions
  - Produce biofilm
- Remove foreign body to provide cure

### Common infections
- Subacute bacterial endocarditis
- Meningitis
- Bacteremia / Septicemia
- Wound infections
- Urinary tract infections
- Peritonitis
- Post-operative infections

### Culture interpretation
- Normal skin flora
  - Common contaminant
    - Wound or Blood cultures
  - Improper collection techniques
- Correlate with other culture and laboratory findings
**Staphylococcus epidermidis**

**Antibiotic Therapy**
- More resistant than *Staph aureus*
- Susceptibility testing done if presumed pathogen
- Drugs of choice
  - Methicillin
  - Vancomycin (if methicillin “R”)

**Staphylococcus saprophyticus**

**Biochemical Characteristics**
- Coagulase -
- Mannitol variable
- DNase –
- Novobiocin “R” (<16 mm)

**Staphylococcus saprophyticus**

**Clinical Significance**
- Urinary tract infections
  - Cystitis in young women
  - Frequency - 2nd to *E. coli*

**Staphylococcus saprophyticus**

**Antibiotic Therapy**
- Susceptibility testing not routinely done
- Lack correlation between *in vitro* and *in vivo* response
- Drugs of choice
  - Nitrofurantoin
  - Trimethoprim/sulfamethoxazole
  - Fluoroquinolone

**Staphylococcus lugdunensis**

**Biochemical Characteristics**
- Latex agglutination +
  - Positive but clumpy
- Tube Coagulase –
- Novobiocin “S”
- PYR +
- ODC +
- Rapid Ornithine →

**Staphylococcus lugdunensis**

**General Info**
- *Staph lugdunensis*
  - PYR positive versus *S. aureus* would be negative
  - ODC positive versus other coag neg staph would be negative
**Staphylococcus lugdunensis**

**Clinical Significance**
- Endocarditis, septicemia, meningitis, skin and soft tissue infections, urinary tract infections and septic shock
  - Particularly aggressive endocarditis
  - Often requiring valve replacement

**Staphylococcus lugdunensis**

**Clinical Significance**
- Susceptibility testing
  - Susceptibility breakpoints for *Staph. lugdunensis* differ from other Coag neg staph
  - *Staph. lugdunensis* uses same breakpoints as *Staph. aureus* for oxacillin
  - Important to identify to the species level for accurate antimicrobial susceptibilities
  - May possess mecA gene

**Micrococcus species**

**General information**
- Normal flora of skin and mucous membranes
- Obligate aerobe
- May possess carotenoid pigments
- Non-motile and non-sporeforming

**Micrococcus species**

**Gram Stain**
- Large GPC in pairs, tetrads or masses

**Micrococcus species**

**Identification**
- Colony morphology
  - Smooth, raised, opaque
  - White, bright yellow, or pink

**Micrococcus species**

**Biochemical Characteristics**
- Tests used to differentiate from *Staphylococcus* species
  - Glucose oxidizer
  - Modified oxidase +
  - Bacitracin sensitive

*Photo courtesy of WEB CLS*
**Micrococcus species**

**Clinical Significance**
- Rarely produces disease
- Normal flora
- Opportunistic infection
  - Immunocompromised

**Micrococcus species**

**Antibiotic Therapy**
- Test methods and therapeutic guidelines do not exist
- Susceptible to most beta-lactam antimicrobials

**Micrococcus**

**Staphylococcus species**

- Gram positive cocci
- Catalase = Positive
- Bacitracin = Sensitive
- Mod. Oxidase = Pos
- Glucose Oxidizer
- Gram Positive cocci
- Catalase = Positive
- Bacitracin = Resistant
- Mod. Oxidase = Neg
- Glucose Fermenter

**In review…….**

**General Information**
- Colony morphology very important
  - Gives us clues for testing needed
- First step in identification
  - Catalase test

**Key points quiz…….**

**Day 4**

<table>
<thead>
<tr>
<th>Student lab</th>
<th>Organism</th>
<th>Atmosphere</th>
<th>Gram Stain morphology</th>
<th>Additional info</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 4 quiz</td>
<td>Staphylococcus aureus</td>
<td>Faculative anaerobe</td>
<td>Gram + cocci in cl/pct</td>
<td>Catalase + Coagulase + Beta hemolytic</td>
</tr>
</tbody>
</table>