Gram-Positive Bacilli

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CLS 418 Clinical Microbiology I
Student Laboratory Session

Differentiation of Major GPR Genera

- Gram stain morphology and arrangement
- Spore formation
- Catalase reaction

Gram-positive rods

- Large w/spores
- "regular-shaped"

Gram-positive rods

- Pleomorphic, palisades of parallel cells, "V" or "L" shapes
- Branching (specimen GS)

Gram-positive rods

- Spore-forming, Catalase +

**Bacillus species**

- Widely distributed in nature
- In clinical specimens considered:
  - Environmental contaminants or normal flora
  - *Bacillus anthracis* is etiologic agent anthrax
  - Opportunistic infections: immunosuppressed

Gram-positive rods

- *Bacillus* species – only aerobic spore-forming GPR
- Capsules (*B. anthracis*)
- Exotoxins – (*B. anthracis* & *B. cereus*)

CL5 418 Clinical Microbiology I
Student Laboratory
Gram-positive Rods
Gram-positive rods

**Spore-forming, Catalase +**

- *Bacillus* species – only aerobic spore-forming GPR
  - Spores
    - Produced when organism stressed
    - Appear clear on Gram stains
  - In vitro induction of spore production
    - Heat shock

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**Bacillus species**

- Organism may stain gram-negative or gram-variable
  - 3% KOH Test: emulsify & raise loop
    - GNR = viscous thread
    - GPR = no thread
  - In vitro induction of spore production
    - Heat shock
  - What can you see on a Gram stain to confirm an organism is a *Bacillus* sp.? Spores

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**Gram-positive rods – spore-forming, catalase +**

- Grow on SBA or CHOC, 24 hrs., ambient air or CO₂
- Identification
  - Usually rule out *B. anthracis* & report as *Bacillus* sp.
    - **Beta-hemolytic**
    - Motile

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**Listeria monocytogenes**

- Identification
  - **BAP**: 24 hrs (growth is small), ambient air or CO₂;
  - Beta-hemolytic (small zone)
  - Gram stain: small GPR
  - Catalase positive

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**Gram-positive rods – spore-forming, catalase +**

- **Listeria monocytogenes**
  - Bacteremia/meningitis immunosuppressed
  - In utero passed to fetus: systemic infection & stillbirth
- Transmission
  - Ingest contaminated food: luncheon meats, dairy products
  - Will grow at refrigerator temperatures
Listeria monocytogenes

• Identification
  – Can be confused with Group B Streptococcus

What rapid biochemical test will differentiate Group B Strep and *Listeria monocytogenes*?

**Who Am I?**

**Isolated from an arm wound**

Catalase: bubbles produced when hydrogen peroxide added to the organism (on a glass slide)

Bacillus species

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*Corynebacterium* species

• Many species are normal flora of skin & mucous membranes
• Most species are nonpathogenic
• Referred to as “diphtheroids”
  – Palisading GPR
  – Catalase positive

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*Corynebacterium* species

• Isolation
  – BAP: growth 24 hrs, ambient air or CO₂
  – Various colony morphologies: dry, irregular shaped, white to buff colored
• Loeffler’s methylene blue stain (Albert’s stain) = positive

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Clinically Significant *Corynebacterium* species

• *Corynebacterium diphtheriae* – diphtheria
• Humans are only host
  – If cultured from a healthy person, considered a carrier (not considered normal flora)
• Respiratory or cutaneous forms
Corynebacterium diphtheriae

- Respiratory:
  - Organism in upper respiratory tract and forms a pseudomembrane (WBCs & organism)
  - Pseudomembrane should be cultured
  - May cause respiratory obstruction
- Exotoxin produced, bloodstream, acts on cardiac tissue & peripheral nervous system
- Mortality 10-30% due to congestive heart failure

Pseudomembrane (WBCs & organism) should be cultured

Corynebacterium diphtheriae

- Cutaneous – nonhealing ulcers
- Treatment – antitoxin given in form of toxoid (detoxified antitoxin)
- Prevention – DPT immunization

Culture & Isolation – Specific request
- BAP: 24-48 hrs; small gray translucent to medium, opaque white colonies
- Loeffler’s media: stimulates growth & formation of metachromatic granules
- Cystine-tellurite: grayish-black colonies
- Modified Tinsdale: black colonies w/dk brown halo

Cystine tellurite: grayish black colonies

Modified Tinsdale: black colonies w/dk brown halo

Gram stain

CHO utilization: Glucose + Sucrose (-)

Urea (-)

Nitrate Reduction variable
GPR: irregular, nonspore-forming, catalase +

*Corynebacterium diphtheriae*

• If id as *C. diphtheriae*, then must determine if isolate is an exotoxin producer (i.e., can cause disease)
  – In vivo test (guinea pigs)
  – Immunodiffusion – ELEK
  – Tissue culture neutralization
  – PCR

GPR: irregular, nonspore-forming, catalase +

*Clinically Significant Corynebacterium sp.*

• *Corynebacterium jekeium*
  – Probably of low virulence
  – Immunocompromised patients: septicemia, wounds, endocarditis, pulmonary disease
  – Often patient has intravenous catheter
  – RESISTANT to many of the antibiotics used to treat gram-positive infections
• Clue: multiple specimens with predominance of "diphtheroid-like" GPR

GPR: irregular, nonspore-forming, catalase +

*Corynebacterium jekeium*

• Isolation & Identification
  – BAP: nonhemolytic, small white to gray colonies, may take 48-72 hrs for good growth
  – GS: like other diphtheroids
  – Very inert
    • Lipophilic
    • Glucose "O", Maltose is variable
    • Resistant to antibiotics used to treat GP infections, Vancomycin susceptible

GPR

Review – Aerobic (Facultative) GPR

• Identification
  1. Gram stain & catalase reaction
  2. Colony morphology & growth characteristics
  3. Determine level of id needed (NF vs. pathogen) based on site and patient
  4. Definitive biochemicals if needed

Who Am I?
Isolated from an arm wound

Catalase positive

Possible *Bacillus anthracis*: further id needed (send to reference lab & report)
**Mycobacterium sp.**

- *Mycobacterium tuberculosis*
- *Mycobacterium avium complex* (*MAC attack* HIV+ individuals)
- Aerobic, non-spore formers
- High lipid content in cell wall – doesn’t Gram stain well
  “beaded” GP appearance

**Mycobacterium sp.**

- Acid-fast – the organism resists decolorization with acid-alcohol or acid-acetone solutions
- Carbolfuschin stains (Ziehl-Neelsen/Kinyon)
  Primary stain = fushsin (red/magenta)
  Methylene blue = counter stain (blue)

**Mycobacterium sp.**

- Growth conditions/time requirements
  3-8 weeks on solid media
  10-11 days w/liquid systems
- Safety concerns: never work with specimens/isolated w/o working under biological safety hood
  Specific safety criteria
  Specific specimen processing protocols
- Specific culture request