

## ANAEROBIC BACTERIOLOGY: COURSE OUTLINE

Prerequisites: MB 540, and CH518 or departmental permission.

Description: Basic biology of pathogenic anaerobic bacteria. Fundamentals of anaerobic bacteriology and the development of disease with the avian species used as a model. Where appropriate pathogenicity in other veterinary species and humans will be discussed. Basic clinical methodology, anaerobic bacterial identification, pathogenesis and current literature will be dealt in detail. Current literature will be examined in detail.

### I. Introduction

#### A. Defining an anaerobe.

1. How anaerobes differ from aerobic bacteria.
  - a. Basic physiology of anaerobes.
  - b. Substrate-level phosphorylation.
  - c. Electron transport.
  - d. Anaerobic ATP production via respiration.
  - e. Anaerobic ATP production via fermentation.
2. Categories of oxygen tolerance.
3. Basic redox chemistry.
  - a. Measurement of redox potential.
  - b. Electronegative coupling and electron flow patterns.
  - c. Maintenance of redox equilibrium.
4. Oxygen lethality theory.
  - a. Oxygen acting as an oxidant.
  - b. Oxygen acting as a toxic agent.
  - c. Free radical formation and molecular oxygen reduction.
    1. Superoxide anion formation.
    2. Hydrogen peroxide formation.
    3. Hydroxyl radical formation.
    4. Singlet oxygen formation.
5. The role of SODs in bacteria and higher organisms.
  - a. Metalloprotein catalyzed dismutation of superoxide anion.
  - b. Superoxide dismutase theory.
  - c. Two-phase oxygen lethality.

#### B. Anaerobic bacterial ecology.

1. Current literature review.
2. Alimentary tract function.
3. Intestinal contents.
4. Mucosal architecture and its affect on colonization.
5. Intestinal bacteria.
6. Bacterial distribution.
7. Bacterial numbers.

#### C. Disease etiology- a conceptual introduction.

1. Definition of diseased state.

2. Aberration from steady state dynamics.
  3. Local ecology effects.
  4. Physiological affects and interaction with local flora.
- D. Anaerobic bacteriology history.

## II. Disease etiology

### A. Sporeformers.

1. Endospore architecture.
2. Endospore formation.
3. Endospore germination.
4. Current literature review.
5. Bacilli
  - a. Clostridium sp.
    1. Botulinum toxin groups.
    2. Structural components of pre-toxin and nicked toxin stereo-chemistry.
    3. Phage involvement
    4. Intracorporeal toxin production.
  - b. Bacillus sp.

### B. Non-sporeformers.

1. Gram positive.
  - a. Bacilli.
    1. Actinomyces sp.
    2. Arcanobacterium sp.
    3. Bifidobacterium sp.
    4. Eubacterium sp.
    5. Lactobacillus sp.
    6. Odds and ends.
  2. Gram negative.
    - a. Bacilli.
      1. Bacteroides sp.
      2. Campylobacter sp.
      3. Porphyromonas sp.
      4. Prevotella sp.
      5. Infrequent isolates.
    - b. Spirochetes.
      1. Treponema sp.
      2. Borrelia sp.
    - c. Cocci.
      1. Minor species.

Each of the bacteria will be reviewed in terms of the following criteria:

1. Zoonotic potential
2. Pathogenesis
3. Transmission
4. Incubation
5. Signs

6. Lesions
7. Differential diagnosis
8. Reported treatments
9. Biochemical characteristics useful for identification.

Note: Some of the bacteria and bacterial diseases discussed in this section will be from other than avian species. These have been included for familiarization with the genera and because they are better characterized in other species.

### III. Endogenous flora/Exogenous flora.

- A. Primary pathogens.
  1. Etiological development.
- B. Secondary pathogens.
  1. Etiological development.
- C. The "anaerobe scenario".

### IV. Pathogenesis/Virulence Factors.

- A. Gram positive organisms.
  1. Toxins- A primer.
    - a. Enterotoxin
      1. Multi-subunit proteins vs. small heat-stable polypeptides.
      2. Clostridium perfringens toxins in depth.
        - a. CPE spore coat structure and function.
        - b. Pathophysiological effects of CPE.
        - c. Cytotoxicity expression.
        - d. Neurotoxin mode of action.
          1. Exocytosis.
          2. Binding, Internalization and axonal transport.
          3. Collagenases.
          4. Clostridium botulinum toxins.
            - a. Toxin types
            - b. Toxin structure and differentiation.
            - c. Genetics/plasmids in C. botulinum toxin expression.
- B. Gram negative organisms.
  1. Capsules.
  2. Adherence.
  3. Other virulence factors.

### V. Anaerobic etiology signs.

- A. The "magnificent seven".

### VI. Selection and collection of anaerobic samples.

- A. Defining acceptable specimens.
- B. Defining unacceptable specimens.
- C. Site selection and preparation.

- VII. Transport of anaerobic specimens.
  - A. Aspirates.
  - B. Swabs.
  - C. Tissue.
  - D. Blood and other fluids.
  
- VIII. Anaerobic specimen processing.
  - A. Macroscopic examination.
    - 1. Colony morphology.
  - B. Microscopic examination.
    - 1. Modified gram stain techniques.
    - 2. Cellular morphology.
  - C. Media selection.
    - 1. Selective media.
    - 2. Non-selective media.
    - 3. The use of PRAS media for strict anaerobes.
    - 4. Specimen techniques.
      - a. Aspirates.
      - b. Swabs.
      - c. Tissue.
      - d. Blood and other fluids.
  
- IX. Media preparation.
  - A. PRAS media.
  - B. Reducing agents and indicators.
  - C. Media containing "Oxyrase".
  
- X. Primary isolation setup.
  - A. Selective media selection.
  - B. Non-selective media selection.
  - C. Solid vs. liquid media.
  - D. Aerotolerance testing setup.
  
- XI. Principles of anaerobiosis.
  - A. Anaerobic induction.
  - B. Anaerobic incubation systems.
    - 1. Anaerobic chambers.
    - 2. Anaerobic jars.
    - 3. Anaerobic bags.
  
- XII. Processing suspect anaerobes.
  - A. Clues to the presence of anaerobes.
  - B. Colony descriptions.
  - C. Colony characteristics.
  - D. Confirmation of gram stain results.
    - 1. Special potency disks.
  - E. Aerotolerance testing.

XIII. Presumptive identification of anaerobes.

- A. Quick tests.
  - 1. Fluorescence.
  - 2. Disk tests.
  - 3. Spot tests.
  - 4. Motility tests.
  - 5. Lecithinase/Lipase reactions.
  - 6. Urease test.
  - 7. Oxidase tests.

XIV. Presumptive identification flow charts.

- A. Anaerobic, gram-positive cocci.
- B. Anaerobic, gram-positive bacilli/coccobacilli.
- C. Anaerobic, "Clostridium-like", gram-positive bacilli.
- D. Anaerobic, gram-positive diphtheroids.
- E. Anaerobic, gram-negative bacilli/coccobacilli.
- F. Anaerobic, Vancomycin-resistant, gram-negative bacilli/coccobacilli.
- G. Anaerobic, gram-negative, bacilli/coccobacilli resistant to Vancomycin, Colistin and Kanamycin.
- H. Anaerobic, gram-negative bacilli/coccobacilli with pitting colonies.
- I. *Fusobacterium* sp.
- J. Anaerobic, gram-negative bacilli/coccobacilli resistant to Vancomycin and Kanamycin, susceptible to Colistin.

Note: Some of the bacteria discussed in this section will be from other than avian species but are included for familiarization with the use of flow charts in identification.

XV. CDC differential media- "Presumpto Plates".

- A. Plate 1 reactions.
- B. Plate 2 reactions.
- C. Plate 3 reactions.

XVI. Definitive identification of anaerobes.

- A. Conventional methods.
  - 1. Hungate technique.
  - 2. Tube techniques.
- B. Minisystems
  - 1. API 20A system.
  - 2. Minitek system.
  - 3. Rapid ID Ana II panel.
  - 4. Crystal ID system.
- C. Metabolic end-product analysis- gas liquid chromatography.
- D. Cellular fatty acid analysis- high resolution GLC.

XVII. Susceptibility testing of anaerobes.

- A. Antibiotic resistance.

B. Criteria for susceptibility testing.

C. Testing methods.

1. Agar dilution.
2. Disk diffusion.
3. Broth dilution.
4. Broth microdilution.
5. Epsilometer techniques.
6. Beta-lactamase testing.

XVIII. Antimicrobial agents.

A. Veterinary medications.

B. Human medications.

XIX. Future directions in anaerobic bacteriology.

Requirements:

Tests:

Test 1: Covers sections I-II. (100 points).

Test 2: Covers sections III-VIII. (100 points).

Test 3: Covers sections IX-XIV. (100 points).

Test 4: Covers Sections XIV-XIX (100 points).

Final: Comprehensive Sections I-XIX. (200 points).

Missed tests require an official excuse. Test must be made up by the next class date upon return.

Exams will consist of multiple choice questions, and essays.

Current literature topic presentation: Subject must be approved by end of first week of class. Presentations will consist of a 30 minute talk (with slides), on a subject approved by the instructor, presented to the class. Presentations will be made starting on Tuesday of the fourth week of class at 6 PM. Two presentations will be made each week until all are complete. Grade will be based on: a) Thoroughness of literature review (100 pts), b) Organization and presentation of the material (50 points), c) Ability to discuss material and answer questions from audience (50 points).

Total 200 points.

Laboratory: (200 points total including 100 points for laboratory exercises and attendance, 50 points for laboratory notebook, 50 points for identification of 5 unknown using presumptive methods ).

Laboratory notebook due at completion of final laboratory session. Attendance and laboratory exercises can not be made up. Because of equipment availability, students may have to work after class hours to complete some exercises.

Schedule: 3 one hour lectures and 2 three hour labs per week.

Required Textbooks: Principles and Practice of Clinical Anaerobic Bacteriology  
P.G. Engelkirk, J.D. Engelkirk, V.R. Dowell  
Star Publishing Company, 1992.

Supplementary readings: Free Radicals in Biology and Medicine, 2nd ed.  
B. Halliwell, J.M.C. Gutteridge  
Clarendon Press, 1993.

Intestinal Microbiology  
B.S. Draser, P.A. Barrow  
American Society of Microbiologists, 1985.

Current literature handouts.

Laboratory Exercises:

Lab 1.

1. Safety Briefing. At the end of this session students will sign a safety SOP. Violation of this SOP may threaten the health and welfare of the student or instructors. It is critical to recognize the seriousness of working with pathogenic anaerobic bacteria and anaerobic bacteriological equipment. Violation of the safety SOP may lead to immediate suspension from this class.

2. Introduction to anaerobic bacteriology equipment. Training in anaerobic chamber use. Emergency procedures. Standards of good laboratory practice.

Lab 2.

1. Oxygen tolerance exercise. Set up: *Clostridium perfringens*, *Bacteroides fragilis*, *Escherichia coli*. Incubate anaerobically in chamber and in anaerobic jar, aerobic, CO<sub>2</sub>, gassed tube incubated in aerobic chamber.

2. Gram Stain exercise with same. Read and record results.

Lab 3.

1. Read and record results from lab 2. Record colony morphology.

2. Re-gram stain new growth. Record results.

3. Identify bacteria grown from Lab 2. Use the Ana system, OneE.

4. Subculture *Clostridium perfringens*, *Bacteroides fragilis*.

Lab 4.

1. Set up primary plates with *Clostridium perfringens*, *Bacteroides fragilis*.

a. Brucella agar

b. BBE

c. KVLB

d. PEA

- e. Thioglycolate backup tube.
- 2. Receive mixed culture
  - Aerobic
    - a. BA
    - b. CA
    - c. MAC
    - d. PEA
  - Anaerobic
    - a. PRAS BRU
    - b. PRAS PEA
    - c. PRAS BBE
    - d. PRAS KVLB

Lab 5.

1. Record Results from Lab 4.
2. Gram stain.
3. Set up EYA with *Clostridium perfringens*.
4. Set up EYA with *Fusobacterium necrophorum*.
5. Subculture *Clostridium perfringens*, *Fusobacterium necrophorum*, purified mixed culture bacteria on BRU/BA.

Lab 6.

1. Record results from Lab 5.
2. Set up CCFA with *Clostridium difficile*.
3. Identify bacteria growth from Lab 5 BRU/BA using ANA, OneE.
4. Subculture the *Bacteroides fragilis* and *Clostridium perfringens*.

Lab 7.

1. Set up *Porphyromonas* spp., *Prevotella* spp., *Clostridium difficile*, *Veillonella* spp. on BRU.
2. Set up *Bacteroides fragilis* and *Clostridium perfringens* on quad plate.

Lab 8.

1. Read results from quad plates.
2. Receive chicken ceca and process on:
  - Aerobic
    - a. BA
    - b. CA
    - c. MAC
    - d. PEA
  - Anaerobic
    - a. PRAS BRU
    - b. PRAS PEA
    - c. PRAS BBE
    - d. PRAS KVLB

Lab 9.

1. Subculture bacteria from Lab 8.
2. Perform Gram stain, read and record results.

Lab 10.

1. Set up *Clostridium ramosum*, *Clostridium clostridioforme*, *Fusobacterium* spp. with special potency disks on BRU.
2. Set *Staphylococcus aureus*, *Peptostreptococcus anaerobius* with SPS disks.
3. Set up *Bacteroides fragilis* with disk.

Lab 11.

1. Read results from lab 10.
2. Set up *Clostridium perfringens*, *Bacteroides fragilis* on BRU for E test.
3. Sample recovery and transport exercise with chicken.

Lab 12.

1. Record results from E test.
2. Discussion of anaerobic susceptibility testing.

Lab 13.

1. State Diagnostic Laboratory Visit

Lab 14.

1. Computer exercise.

Lab 15.

1. Computer exercise.

Lab 16.

1. Receive unknown bacterial mixture.
2. Work on unknown.

Lab 17.

1. Work on unknown.

Lab 18.

1. Work on unknown.

Lab 19.

1. Verify ID with biochemical tests.

Lab 20.

1. Turn in notebook.
2. Group discussion.