Gram positive and Gram negative anaerobic rods

Gram positive spore-forming rods

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Anaerobic commensal flora of man

- the commensal flora of man is largely anaerobic;
- anaerobes are found on all the mucosal surfaces and on the skin

Skin
- the skin is constantly exposed to the air, it still supports a considerable anaerobic microflora
- predominantly 'anaerobic diphtheroids' – propionibacteria
Mouth

• anaerobes are found in:
  – the tonsillar crypts, tongue crypts
  – gingival crevices, and dental plaque
  – although some anaerobic species are found in young infants - the number of anaerobes increases with the eruption of the teeth

Predominant members of the oral anaerobic flora include:
  – Prevotella, Fusobacterium, Peptostreptococcus, Veillonella, and various anaerobic Gram-positive bacilli
Intestine

- the stomach and upper small intestine are normally sterile or contain transient organisms - from food, saliva, and nasopharyngeal secretions
- the terminal ileum resembles the colon with a vast and diverse anaerobic flora - is established by the second year of life
- mostly: Bacteroides vulgatus, B. thetaiotaomicron, B. fragilis
- Clostridia are also found in large numbers
Genitourinary tract

- The normal flora of the vagina is predominantly anaerobic.
- Mostly: lactobacilli, Prevotella, Fusobacteria, and peptostreptococci.
Pathogenesis

• the anaerobic bacteria that cause human infection are almost always derived endogenously from the host's own commensal flora
Collection and transport of specimens for anaerobic bacteriology

- All anaerobic bacteria are sensitive to oxygen.
- B. fragilis and C. perfringens will tolerate 2 to 4% oxygen.
- The best specimens are:
  - Aspirates, pus (in a universal container) or excised tissue.
  - Swabs are less satisfactory; a transport medium should be used.
Treatment of anaerobic infections

Surgical intervention:

• drainage of pus and excision of necrotic tissue - that is required to treat the infection

Prevention of anaerobic infection

• antibiotic prophylaxis for operations – ex. cefuroxime and metronidazole
Antibiotic treatment

Metronidazole:
• it is only active against anaerobes - no activity against aerobes
• most clinically important anaerobes remain sensitive

β-Lactam antibiotics
• many anaerobes are still sensitive to penicillin
• B. fragilis group are resistant to penicillin – the penicillin resistant are also resistant to ampicillin, amoxycillin, and most cephalosporins
• combination of β-lactam inhibitor (clavulanic acid) and amoxicillin → bacteria became susceptible
Clindamycin:
• most anaerobes are sensitive

Chloramphenicol:
• is also highly active against anaerobes – but toxic

Erythromycin, co-trimoxazole, and tetracyclines are effective as well as
Glycopeptides, aminoglycosides and quinolones are ineffective
Fig. 6.5 A decision tree used in the laboratory identification of organisms.
### Anaerobic bacteria

<table>
<thead>
<tr>
<th>Genera</th>
<th>Anatomic site</th>
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<tbody>
<tr>
<td><strong>Gram - negative anaerobes</strong></td>
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<tr>
<td>- Gram - negative bacilli:</td>
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<tr>
<td>- Bacteroides species</td>
<td>Colon</td>
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<tr>
<td>- Prevotella species</td>
<td>Mouth</td>
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<tr>
<td>- Porphyromonas species</td>
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</table>
Bacteroides

- Gram-negative slender rods or coccobacilli
- are member of the normal human intestinal flora
- normal stool contain $10^{11}$ B. fragilis/gram
- infections associated with contamination by the contents of the colon, they may cause peritonitis, abscess
- classification is based on biochemical properties and on characteristic short-chain fatty-acid patterns in gas chromatography
Prevotella

- slender rods and coccobacilli
- are found in infections associated with the upper respiratory tract
- P. bivia, P. disiens occur in the female genital tract
- are found in brain and lung abscesses, in empyema, in pelvic inflammatory disease
Porphyromonas

- take part of the normal human oral flora
- newly named species and species that were previously included in the genus Bacteroides
- Porphyromonas can be cultured from gingival and periapical tooth infections, breast, axillary, perianal and male genital infections
Fusobacterium nucleatum

- cigar-shaped rods
- can produce ammonia and hydrogen sulphide from cysteine and methionine → is an odorigenic organism (halitosis)
- isolated from mixed bacterial infections caused by normal mucosal flora
Acute necrotizing ulcerative gingivitis

• also known as: Vincent's disease, Vincent's angina, Vincent's gingivostomatitis, trench mouth, and fusospirochaetosis

• causative agents: Fusobacterium and Treponema vincenti

• affects the gingiva and buccal mucosa

• symptoms:
  – painful bleeding gums, sometimes with a pseudomembrane, and foul breath
Veillonella

- Gram-negative cocci
- take part of the normal flora of the mouth, the nasopharynx and intestine
- considered as benevolent bacteria
- they metabolise lactic acid to weaker acids

Species: V. parvula, V. dispar, V. atipyca
Gram-positive anaerobes

• **Gram-positive bacilli**
  - Actynomyces  Mouth
  - Lactobacillus  Vagina
  - Propionibacterium  Skin
  - Eubacterium, Bifidobacterium, Arachnia  Mouth, colon
  - Clostridium  Colon (also found in soil)

• **Gram-positive cocci**
  - Peptostreptococcus  Colon
Actinomyces

Characteristics:

- non-acid-fast, Gram-positive bacterium
- is anaerobic
- may branch in tissues and convert to rod form
- is found as a commensal organism in dental plaque and female genital tract
- causes chronic granulomatous infection → soft tissues
- form sinus tracts to the surface → exudate from these sinus tracts contains hard microcolonies called granules
Diseases

- **cervicofacial actinomycosis** (lumpy jaw) → after dental work is performed
- **thoracic actinomycosis** → lung and ribs
- **abdominal actinomycosis** → starts in the ileocecal region and frequently produces sinus tract to the skin surface
- **mycetoma** → infection of the limb

Treatment:

- surgical drainage of necrotic tissues
- penicillin
Propionibacterium

• highly pleomorphic
• metabolic product is propionic acid
• are members of the normal flora of the skin
• participate in the genesis of acne
• cause disease when they infect plastic shunts and appliances
• main intraoral sites and infections: root surface caries, plaque biofilms. Possible involvement in dentoalveolar infections
• sometimes contaminates blood or cerebrospinal fluid
Eubacterium

- pleomorphic, Gram-variable rods or filaments
- main species: Eubacterium brachy; E. nodatum; E. saphenum
- main intraoral sites and infections: plaque biofilms and calculus; implicated in caries and periodontal disease
- E. yurii is involved in 'corn-cob' formation in dental plaque
Lactobacillus acidophilus

Cultivation: Rogosa culture media

- take part of normal flora of oral cavity, female genital tract and intestine
- homofermentres – lactic acid (L. casei)
- heterofermenters – lactic acid, etanol, acetate, CO$_2$ (L. acidophilus)

Sugar – lactic acid – damage of enamel - caries
• **In vagina as normal flora** (Döderlein rods)

• **Produce acid** – barrier against causative agents

• **Lactobact** – liophilised L. strains
Lactobacillus acidophilus
Microbial test in caries assessment

• Calculation of L. acidophilus and S. mutans count in saliva-, plaque – or interdental sample

• Dilutions of sample were spreading on selective media -- colonies forming unite /CFU/ are quantified and calculated for 1ml saliva

• High caries activity:
  Lactobacillus CFU 100,000
  S mutans CFU 1,000,000
Bifidobacterium

- 8 species: B. dentium
- Morphology: G+ rods, filamentous form
- Culture: blood agar, anaerobic
- Normal flora in oral cavity, intestinal tract of babies, colonisation resistance Probioticum
- Disease: caries? periodontitis? pathogen in immununsuppressed state
Eubacterium

• Most important member: E. lentum
• Morphology: G+ pleomorphic rods
• Culture: blood agar, anaerob
• Disease:
  – Parodontitis
  – subgingival plaque, dentin caries, pulpitis
  – carbunculus, empyema, endometritis
  – peritonitis, pelvic abscess
CAPNOPHILIC PATHOGEN AGENTS IN ORAL CAVITY
Actinobacillus actinomycetemcomitans

- Normal flora in oral cavity
- Often isolated together with Actinomyces sp.
- Morphology: G-negative coccobacillus
- Culture: blood agar, CO$_2$ media
- Virulence faktor:
  - Leucotoxin, IgA protease, collagenase

Disease:
- Aggressive parodontitis, destructive periodontitis
- Abscess, osteomyelitis
Eikenella corrodens

- **Morphology:** G-negative coccobacillus
- **Culture:** blood agar, CO$_2$ atmosphere
- **Normal flora of oral cavity**

- **Disease:** pyogen infections in oral cavity, gingivitis, and extraoral abscesses
Capnocytophaga

Morphology: Gram-negative rod
- most important species: C. gingivalis

Culture: blood agar, CO$_2$ atmosphere
- colonies spread on the surface of the agar
- Normal flora of subgingival places

Disease: gingivitis, parodontitis, osteomyelitis
Bacillus, Clostridium
### Gram-positive spore forming rods

#### Aerobe
- **Bacillus**
  - B. anthracis
  - B. cereus

#### Anaerobe
- **Clostridium**
  - C. botulinum
  - C. perfringens
  - C. difficile
  - C. tetani
Bacillus anthracis

Morphology:
- non flagellated rod
- 4-5μm
- form short chains (cane or bamboo)
- spore formation in environment
- spore formation at 15°C
• spores to survive in the soil
• biological warfare experiments:
  – on Gruinard Island off the western coast of Scotland
  – explosive release of spores in 1942 and 1943 resulted in soil contamination
  – that persisted until 1986 - formaldehyde decontamination was successfully accomplished
Cultivation:
• aerobe
• at 12-45°C
• the colonies are big and irregular
• no haemolysis on blood agar
**PATHOGENESIS**

- germination of the spores at the entrance – hemorrhagic inflammation in tissues – forming oedema

**VIRULENCE FACTORS**

- two plasmids - encode the toxins and capsule synthesis genes
- capsule (poly-D-glutamic acid – antiphagocytic)
- Exotoxin production
  - lethal factor
  - oedema factor
  - protective antigen
DISEASE
- anthrax

SOURCE OF THE INFECTION
- domestic and wild herbivores (sheep, goats, cattle, water buffalo, antelopes, elephants, giraffes, and zebras) – are sensitive
- carrion birds and mammalian predators (jackals, hyenas, and lions) - are resistant
- butchering meat, harvesting the hide, or necropsy - increases the quantity of spores in the environment
Countries with cases of human infection with *Bacillus anthracis* - Global distribution
• humans become incidentally infected:
  – when contacting spores on dead animals or their meat, hides, hair, or wool

• by skin contact - cutaneous anthrax
• by consumption - oral-oropharyngeal
• by inhalation – pulmonary
• by enteral pathway - gastrointestinal
Cutaneous anthrax

- after an incubation period of 1 to 7 days, local pruritus
- within a day, forma a papule
- on the second day, one or more vesicles
- the papule ruptures, forming an ulcer
- eschar formation – painless, no pus
- other clinical manifestations: regional lymphadenopathy and nonpitting edema around the eschar
Oral-Oropharyngeal and Gastrointestinal Anthrax

- eating raw or slightly cooked meat
- incubation time 2 days
- the lesions, edema and necrosis - by the end of the first week
- early in the second week a pseudomembrane forms over the ulcer
- symptoms: swelling of the neck, fever, sore throat, and dysphagia
- gastrointestinal anthrax: gaseous distention, air-fluid levels, and ascites, abdominal pain, nausea, vomiting
Inhalational anthrax

- contaminated wool, hair, or other animal products, or as bioterrorism
- spores are transported to the mediastinal lymph nodes
- where they germinate, replicate, and secrete toxins
- that cause massive hemorrhage, edema, and necrosis in these nodes
PREVENTION
• in Hungary: only domestic animals by spore (live attenuated) vaccine - contain the plasmid for production of protective antigen, lethal factor, and edema factor but lack the plasmid with genes for synthesis of the capsule
• Human vaccines: a live spore vaccine
• prophylactic antibiotics are ineffective in preventing inhalational anthrax unless given for 6 days

THERAPY
• penicillin, tetracycline, ciprofloxacin, erythromycin
Saprophytic bacilli

Can cause very rare diseases

B. cereus:

Characteristics:
- big rod in chains
- irregular, huge colonies - motile
- no capsule
- cause beta-haemolysis
DISEASES

• food poisoning by two enterotoxin
  – 2 form: emetic form after 1-6 hours
diarrheal form after 10-24 hours

DIAGNOSIS

- in food - $10^5$/g B. cereus and toxins

THERAPY: self limited disease, rehydration

B. stearothermophilus – biological test of autoclaves, hot air sterilizers
CLOSTRIDIUM TETANI

INTRODUCTION:

- 1 million cases of the disease occurred annually in 1975
- neonatal tetanus having an up to 90% mortality rate
- in the 18th century neonatal tetanus was known as the "7-day disease" in the Americas
- "9-day fits in Dublin" because it usually presents about 1 to 2 weeks postpartum
• Estimated neonatal tetanus mortality rates, 1991 (per 1000 live births).

- fewer than 1
- 1-5
- more than 5
Reported annual incidence of all tetanus cases (including neonatal) by tropical region for years 1990 to 1995
MORPHOLOGY

- Gram positive slender rod
- Motile, peritrichous flagellated
- Terminal spores – drumstick shape
- Spores can survive indefinitely

CULTIVATION

- Obligate anaerobe
- Holman, Brewer culture media - 3-4 days

ANTIGENIC STRUCTURE

- O and H antigen
- By flagella 10 serotype (the toxin is the same)
- Toxins: tetanospasmin and tetanolysin
PATHOGENESIS

• tetanus (spastic paralysis) caused by tetanospasmin

• tetanolysin: may aid in the damage of viable tissue near the wound site, lowering the redox potential?
PATOMECHANISM

- spores entering at skin injury - by soil, during giving birth, abortion, burnt wound, infection of navel
- bacteria will multiply in wound in death tissues
- no bacteraemia – only toxaemia

Tetanus is classified into four clinical subtypes:
- generalized
- localized
- cephalic
- neonatal
SYMPTOMS

- The length of the incubation period depends on the distance of the inoculation from the CNS.
- Trismus or lockjaw - rigidity of the masseter muscles.
- Tetanic spasms last for a few seconds to minutes.
- Spasms are extremely painful.
- The disease may continue to progress for 10 to 14 days.
- Without antitoxin, the disease persists for as long as the toxin is produced.
THERAPY
- removing the tissue debris
- giving antitoxin - HTIG
- giving tetanus toxoid
- penicillin
- giving relaxants
- ventilatory, nutritional, and general ICU support

PREVENTION
• toxoid in DPT
**Clostridium botulinum**

**CULTIVATION**
- on blood agar

**ANTIGENIC STRUCTURE**
- O and H antigen
- 8 type of toxins A-H
- the A, B, E is human pathogen

**PATHOGENESIS**
- Botulisms
- home-canned vegetables, fruits, and fish products
- the spores in food will germinate – toxin
- production – the toxin will inhibit the releasing of the neurotransmitters
- 1-2 ng is fatal
- flaccid paralysis
Symptoms:
After hours or days:
• Paralysis
• difficulties at swallowing
• blocking the respiration system
• gastroenteral symptoms may occur
Infant botulism

- in the first 6 months of life
- in digestive system the spores will germinate and multiply
- will start to produce toxin
- most common sources: honey
DIAGNOSIS AND TREATMENT

- toxin detection from food, vomiting, sera
- inoculation in animal
- toxin detection from sera by latex agglutination
- giving polyvalent sera (A, B, E)
- giving antitoxin
- artificial ventilation
Clostridium difficile

- was first noted in the 1950s
- after antibiotic treatment in the intestine will start to overgrow the poliresistant strains
- ampicillin, clindamycin, and cephalosporins - most common
- clindamycin appears to have the highest relative risk

Toxin production:
toxin A: enterotoxin
toxin B: cytotoxic
toxin A: pseudomembranous colitis (damage of the epithelium, microabsceses)
SYMPTOMS
• bloody diarrhoea, dehydration, anorexia
• abdominal cramps
• fever

DIAGNOSIS
• toxin detection from faeces by latex agglutination

THERAPY
• metronidazol
• vancomycin
Gas gangrene clostridia

C. perfringens – 90%

C. novyi

C. septicum

Pathogenesis:
- multiplication in smashed tissue (anaerobe)
- exotoxin production

C. perfringens (A-E type)
capsulated, non flagellated

Pathogenesis:
A type gas gangrene
Incubation time: 1-3 days

Spreading by wound injuries – muscle – tissue necrosis – forming oedema – gas production

Toxaemia

alfa-toxin

cause haemolysis

cause necrosis
Enzymes:
- collagenase
- hialuronidase
- proteinase
- DN-ase

Therapy:
- antitoxin, Penicillin

Food poisoning:
- A-type – diarrhoea, vomiting, fever – self limited

Enteritis necrotisans:
- C-type: by food which is infected by pig faeces – necrosis of the intestine – perforation - death