Anaerobic bacteria (strict or obligate anaerobes)

- viability is determined by the state of oxidation of the environment they are in
  - state of oxidation expressed in terms of oxidation–reduction or redox potential
  - require complete removal of oxygen for
    a. viability b. metabolism c. multiplication
- exposure to oxygen leads to biochemical reactions with by-products which are toxic to the organisms
  - toxic by-products include
    a. negatively charged superoxide radical O_2^-
    b. hydrogen peroxide

- obtain energy through fermentative pathways
- lack some properties which are possessed by strict aerobes and facultative anaerobes including
    1. cytochrome system
    2. enzymes a. catalase b. peroxidase and c. superoxide dismutase
        - **superoxide dismutase** catalyzes the conversion of superoxide radicals to hydrogen peroxide and oxygen
        - **catalase** breaks down hydrogen peroxide to water and oxygen
- degree of intolerance to oxygen varies among the anaerobic bacteria

Examples of anaerobic bacteria associated with human infections

- classified according to
  - a. shape b. Gram's stain c. spore-formation
    1. bacilli
  - a. Gram positive non-spore formers include genera Actinomyces Eubacterium Bifidobacterium Propionibacterium
  - b. Gram positive spore-former Clostridium
  - c. Gram negative non spore formers Bacteroides Prevotella Fusobacterium

2. cocci

   a. Gram positive include genera
      Peptococcus Peptostreptococcus

   b. Gram negative genus Veillonella

3. spirochaetes

   . specific species and strains of Treponema
      and Borrelia

Occurrence of strict anaerobes

- generally
  a. gastrointestinal tract of animals
  b. environment soil water sewage
- in humans
  a. normal flora in the
     - mouth
     - intestine particularly the colon
     - lower parts of genitourinary tract
     - selected areas on the skin
  b. in various tissues in association with disease

Laboratory techniques for detection of strict anaerobes

I. **Staining and microscopy**
   - Gram's stain and microscopy for most organisms

II. **Gas liquid chromatography** of fluids for detection of products of metabolism including
   a. butyric acid
   b. propionic acid
   c. oxybutyric acid

III. **Special culture techniques**
   - variety of methods improvised or designed to exclude air or oxygen from growth environment

Culture methods for strict anaerobes in the laboratory

specimens

- higher chances of isolation from specimens of
  1. various infected sites in internal organs or tissues and body cavities and fluids including
     a. aspirates or fluids from lungs
     b. discharge or pus from abscesses
     c. infected tissues
  2. infected tissues or fluids from wounds contaminated with dirty material including soil
- collected transported and processed applying necessary care to increase chances of isolation
  - by minimizing chances of contamination and exposure to air

culture media for isolation of strict anaerobes include

1. Blood agar (BA) suitable for most pathogens
2. selective medium BA containing antibiotics
3. Robertson's Cooked Meat medium (RCM)
   a. for initial inoculation of specimens which may contain strict anaerobes in small numbers to enhance their multiplication
      - inoculated and incubated for 72 hours then sub-cultures made on BA and incubated appropriately
   b. In propagation of strict anaerobes in the laboratory
4. Thioglycollate medium contains reducing agent sodium thioglycollate

incubation methods of cultures for strict anaerobes

- improvised techniques to ensure complete elimination of air in the incubation environment include

1. anaerobic jar techniques mainly
   a. Fildes-McIntosh or McIntosh-Fildes jar
      - evacuation of air and replacement with a mixture of inert gases including CO_2
   b. GasPak or Oxoid jars
      - chemicals in gas generating kits react with water to generate a mixture of gases
   - traces of oxygen in a) and b) is removed by a reaction with hydrogen catalyzed by palladium to form water
2. other incubation methods

Clostridium

- most species are flagellated and motile
- form spores
  - diameter larger than the width of the cell
  - centrally or sub-terminally or terminally placed in the bacillus
  - resistant to adverse physical conditions
    - enable prolonged survival of the organism in the environment

Species of Clostridium

- normal flora in the large intestinal tract of animals including humans and saprophytes in
  a. soil b. water c. decomposing animals and plants
- species associated with diseases in humans include
  C. perfringens or *C. welchii* *C. tetani*
  C. botulinum *C. difficile* *C. histolyticum*
- species which may be isolated from specimens but not involved in disease causation is *C. sporogenes*

Main pathogenicity properties of Clostridium species

- most pathogenic species possess the ability to
  1. form spores
     - spores increase the chances of survival and enhance transmission
  2. retain viability and multiply in tissues without oxygen or with reduced blood supply
  3. produce exotoxins
     - main virulence factors responsible for major clinical manifestations of associated diseases
     - composition and mechanisms of activity differ among the species
- specific pathogenic species produce aggressins and other enzymes

Clostridium perfringens or *C. welchii*

- contaminates the environment via faeces from humans and other animals
- spores are
    a. oval and centrally placed
    b. formed in the intestines and environment
- capsulated in tissues
- non-motile

Significant biochemical properties include

- predominantly saccharolytic
- mildly proteolytic
- nitrate reduction test positive

Antigens of *C. perfringens*

- releases different antigens designated A B C D E
- basis for classification into corresponding serotypes

Virulence products of *C. perfringens*

1. Exotoxins

- several different exotoxins released by the serotypes
  - responsible for tissue damage and severity of disease manifestations
  - each serotype releases more than one exotoxin
  - each type of toxin
    a. can be released by different serotypes
    b. has its specific mechanism of activity

exotoxins of *C. perfringens* include

a. alpha (α) toxin
   - released by all serotypes
     - major toxin of *C. perfringens* serotype A
     - relatively heat stable
     - enzyme phospholipase or lecithinase
       - splits lecithin which is a constituent of cell membranes

b. enterotoxin
   - released by specific strains

2. other virulence products *C. perfringens*
   - include hyaluronidase

Clinical implications of *C. perfringens*

- associated with several conditions including
  a. gas gangrene
  b. wound and other soft tissue infections
  c. food related gastroenteritis or food poisoning
  d. infections involving pelvic tissues in adult females
  e. intra-abdominal infections

Gas gangrene or clostridial myonecrosis

- severe condition characterized by
  a. rapidly spreading swelling
  b. necrosis of tissues at the site
  c. myositis
  d. gangrene and evidence of gas in involved tissues

- conditions predisposing to gas gangrene include
  1. traumatic injury involving skeletal muscle
     - associated with the majority
  2. abdominal injury involving the large intestine and leading to leakage of intestinal contents from the lumen to adjacent tissues
- causative organisms are mainly species of Clostridium as single species or more than one
  - most common *C. perfringens* and more frequently serotype A
  - other species including *C. septicum* *C. histolyticum* *C. novyi*

- facllitating factors in development of gas gangrene

1. injury which causes
   a. open wound and damaged skeletal muscle exposed to contamination with dirty material containing bacterial spores
   b. impairment of normal blood flow leading to reduction in oxygen tension in the injured part
   c. devitalization of tissues
   . b) and c) create a suitable condition for viability and multiplication of *C. perfringens*

2. spores germinate into bacilli which multiply and release exotoxins which act on the skeletal muscle
   - causing destruction associated with gas formation
   - alpha toxin is the major cause of the associated tissue damage and manifestations

Food-borne gastroenteritis or food poisoning by *C. perfringens*

- associated with food contaminated by specific strains
  - form spores that survive boiling for several hours
  - spores germinate into bacilli in the food
  - bacilli are ingested as the food is consumed
  - bacilli produce enterotoxin in the intestines
- manifests as mild abdominal cramps and diarrhoea 8 to12 hours after ingestion
  - fever and vomiting are not common
  - illness subsides within 24 to 48 hours
- isolation and identification of the specific strains from stool and suspected food may be useful in laboratory confirmation where necessary

Other *C. perfringens* infections

Infections associated with female reproductive system

- tend to occur as complications after child birth or traumatic abortion
- organism from the large intestine is transmitted to devitalized tissues through contamination directly or via items used

Intra-abdominal infection

- may result from local spread from intestinal lumen to adjacent tissues in association with other abnormalities

Wound and other soft tissue infections

- encountered as inflammatory processes without involvement of skeletal muscle
    - different conditions from gas gangrene

Laboratory investigation of gas gangrene and other infections by *C. perfringens*

specimens from infected sites include
swabs or fluid from wounds or necrotic tissue

laboratory procedures

1. Gram's stain of smears for Gram positive bacilli
2. culture for isolation and identification

media
   a. BA
   b. RCM
      - inoculated with a portion of the specimen
      - incubated under anaerobic conditions for approx 2 to 3 days
      - sub-cultures are made on BA

incubation as described for anaerobes

- temperature of 35 to 37 degrees c for 48 hours

colonies of *C. perfringens* on BA

- relatively large translucent smooth surface
- double zone haemolysis
    - inner zone of complete haemolysis and a larger outer zone of incomplete haemolysis
- microscopy shows Gram positive bacilli
- spore stain after prolonged incubation

3. demonstration of alpha toxin production
    - presumptive identification test
    - methods including Nagler test or Nagler reaction

Management of gas gangrene

A. specific mainly
   1. surgical management of wounds
   2. antibiotics
      - effective agents include penicillin and metronidazole

B. non-specific supportive measures

Prevention of gas gangrene methods include

1. proper care of wounds in general by cleaning and removal of foreign materials and dead tissue
2. proper cleansing of skin before invasive procedures and use of sterilized instruments and materials
3. antibiotic prophylaxis in management of fresh or trauma associated wounds