Microorganisms Causing Foodborne Disease

Gram-positive sporeformers
- *Clostridium perfringens*
- *Clostridium botulinum*

**Clostridium botulinum**
- Strictly anaerobic
- Gram-positive
- Sporeform
- Causes botulism
  - Severe intoxication
  - Flaccid paralysis caused by a neurotoxin
- 8 toxins described
  - A, B, C, C₁, C₂, D, E, F, G
    - C₂ is not a neurotoxin
    - These toxins are heat labile (80°C for 5 min)

*C. botulinum*

Botulism
- 1st outbreak described in 1793 in Germany
  - Associated with sausage
    - Latin *botulus* = sausage
- There are 3 categories of botulism
  - Food poisoning
    - Associated with home-made canned foods or preserves
  - Intoxication
  - Wound botulism
    - Associated with handling contaminated materials when being wounded
    - Toxinfection
  - Infant botulism
    - Associated with bee honey in lactants
    - Toxinfection
**C. botulinum**

Food poisoning

- Incubation period of 12 to 36 hours
- Begins with gastrointestinal problems:
  - Nausea
  - Vomiting
  - Diarrhea
    - May not be caused by the toxin because not present in other illnesses
    - When typical botulism signs develop, constipation
- First indicators of botulism:
  - Fatigue
  - Muscular weakness

**C. botulinum**

- Followed by ocular effects:
  - Droopy eyelids
  - Sluggish response of pupils to light
  - Blurred and double vision
- Mouth effects:
  - Dryness
  - Difficulty in speech and swallowing
- Musculatures controlling limbs and respiration become progressively paralyzed
- Death within 3 to 5 days (respiratory failure)
- Complete recovery may require several months

**C. botulinum**

- Epidemiology
- Most cases are caused by types A, B or E
- *C. botulinum* Type A most frequent in the U.S.
  - Associated with fruits and vegetables
- *C. botulinum* Type E is a psychrotroph
  - Associated with smoked fish
- *C. botulinum* Type B more frequent in Europe
  - Associated with home-canned ham or baked foods
- Commercially canned foods have only been associated with few outbreaks
- Sausage has not been associated with botulism in decades
Methods for isolation

- Needs anaerobic environment to grow
- Food suspension is heated to eliminate non-sporeformers
- Enrichment in cooked meat broth at 30°C for 7 days
- Plate on horse blood or egg yolk agar
- Select typical colonies
- Conduct toxin identification

**C. perfringens**

- Gram positive
- Sporeforming rod
- Strictly anaerobic
  - Will survive and occasionally grow in the presence of O₂
- Encapsulated
- Nonmotile

**C. perfringens**

- Formerly *C. welchii*
- Associated with gas gangrene
- One of the most common causes of diarrhea worldwide
- 5 types based on toxin production
  - A, B, C, D, E
  - Toxins α, β, ε and 4
- *C. perfringens* type A is responsible for food poisoning and gas gangrene
  - Only produces α toxin
  - This toxin also has lecithinase activity
  - Phospholipase
- *C. perfringens* type C
  - Produces α and β toxins
  - Cause of enteritis necroticans
  - Severe but rare condition associated with pork in Papua New Guinea during festivities
**C. perfringens**

- Growth characteristics (vegetative cells)
  - Temperature
    - 12 – 50°C
    - Optimum 43 – 47°C
      - Generation time of 7.1 min at 41°C
  - pH
    - Minimum 5.0
    - Optimum 6.0 – 7.5
    - Min. $a_r$ 0.95 – 0.97
- Resistance (spores)
  - $D_{100} = 0.31 – 38$ min

**C. perfringens**

- Incubation period = 8-24 h
- Necessary to ingest large numbers of vegetative cells ($10^6 – 10^8$ cells)
- Syndrome
  - Explosive diarrhea
  - Severe abdominal pain
  - Nausea less common
  - Fever and vomiting unusual
  - Death uncommon unless underlying illness
  - Symptoms subside in 12 to 24 h

**C. perfringens**

- Found in most raw, animal products
  - Raw meats
  - Raw poultry
  - Dehydrated soups and sauces
  - Raw vegetables
  - Food ingredients (spices)
  - Virtually all processed foods
- Foods associated with outbreaks:
  - Cooked meat or poultry foods
    - Stews
    - Meat gravy
    - Meat pies
    - Roast beef
- Why these foods?
  - High prevalence
  - High nutrient content (protein)
  - Cooking creates reduced environment suitable for growth
**C. perfringens**

- Outbreaks frequently occurring at food-service establishments
- Temperature abuse
- Spores need to germinate and grow to high numbers to cause infection
- Once the intestine is colonized, vegetative cells sporulate
- Toxin is released at sproulation

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**C. perfringens**

- Control and Prevention
  - FDA Food Code
    - Rapid, uniform cooling of food
      - To 21°C within 2 h, and
      - from 21°C to 5°C within 4 h
    - Reheat to internal temperature of 74°C
    - Hold cooked dishes ≥60°C or ≤5°C
  - Impossible to eliminate from foods
  - Prevent by proper food preparation and storage techniques

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Methods for analysis
- Dilutions and plating onto tryptose sulfite cycloserin (TSC) agar
- Incubate under anaerobic conditions
- Select typical colonies for further identification
  - Reduce NO₃ to NO₂
  - Non motile
  - Lactose fermentative
  - Liquefies gelatin
- MPN is also used when low numbers are expected

C. perfringens