1. **Background**

   - Botulism is a serious illness that causes *flaccid paralysis* of muscles
   - *Clostridium botulinum* produce exotoxin in anaerobic environment
     (Most *Clostridium* species produce only one type of neurotoxin.)
   - Not transmitted from person to person
   - **Botulism develops**: ingestion (inhaled or injected) or organisms grow in the intestines or wounds in the body and toxin is released.
   - There are seven types of botulism toxin designated by the letters A through G; only types A, B, E and rarely F cause illness in humans. (CDC)
   - Today – Nitrites, sorbic acid, parabens, phenolic antioxidants, polyphosphates and ascorbates prevent *C. botulinum* from growing
   - Foodborne botulism is often caused by eating improperly processed food. Homemade canned and bottled, preserved or fermented foodstuffs require extra caution (WHO)

2. **Type A toxin**

   - 60 ~ 70% mortality rate (in untreated case, type A endospore is most heat resistant in the strain)
   - Usually proteolytic, but spoilage order is not present such as in corn and beans
   - 35°C: Optimum temperature for growth and toxin production of proteolytic

3. **Region**: California, Washington, Colorado, Oregon, and New Mexico

4. **Type B toxin**

   - 25% mortality rate
   - Occurs in proteolytic and nonproteolytic
   - 26-28°C: Optimum temperature for growth and toxin production

5. **Region**: Europe and eastern U.S.
• **Type E toxin**
  - Found in marine and lake sediments
  - Involve in fish and other sea food
  - 3-4°C: Nonproteolytic types B, E, and F can produce toxin at refrigeration temperatures
  - **Region**: Pacific Northwest, Alaska, Great Lake area

• **The main limiting factors for growth**
  - Temperature, pH, water activity, redox potential, food preservatives, and competing microorganisms
  - Though mainly a foodborne intoxication, botulism can also be caused by intestinal infection in infants, wound infections, and by inhalation. (WHO)

2. **History of Botulism**

• 1735 : History of botulism begins with German sausage (Food Poisoning)
• 1870 : German physician Muller named botulism from the Latin word for sausage
• 1895 : C. botulinum bacteria were 1st isolated
• 1944 : A neurotoxin was isolated by Dr. Edward Schantz
• 1949 ~ 1950 : The toxin (named BoNT-A) was shown to block the release of Acetylcholine by somatic motor neurons
• 1980 : Dr. Scott used the toxin to treat strabismus (deviation of the eye)
• 1989 : BoNT-A (BOTOX) was approved by the U.S. Food and Drug Administration (FDA) for the treatment of strabismus, blepharospasm, and hemifacial spasm in young patients
• 2002: The FDA approved the uses for cosmetic, underarm sweating, and muscle pain disorders (Botulism neurotoxin is used in dilute concentration to treat medicine and cosmetic condition.)

3. **Cause of Disease**

• The symptoms are caused by the toxin produced by the bacterium.
  - The number of early causes has dropped to about 1000
worldwide, in the U.S. on average 110 cases are reported
- Suffering from **flaccid paralysis** for 1~10 days
  and may die from respiratory and cardiac failure (see pp. 616)
- Toxin blocks the release of acetylcholine
  by somatic motor neurons
- Adult intestinal colonization and iatrogenic botulism
  also occur, but rarely.
- Untreated botulism death rate: about 50%
- Treated patients death rate: about 3%-5%.

  • **Food born Botulism : 25%**
  - Can be poisoned by eating even small amounts of food
  - 18-36 hours after eating a contaminated food
    (six hours or as late as 10 days)
  - Social gatherings or restaurants
  - Mold growth, metabolized acid to allow to begin growing
  - Ready-to-eat foods in low oxygen-packing
  - Serum, feces, vomits, gastric contents and suspected food

  • **Wound Botulism : 3 %**
  - Pathogen grow in wound, black-tar heroin injection
  - Serum, feces, debrided tissue, swap samples from wounds of patients

  • **Infant Botulism : 72 %**
  - Sudden infant death syndrome (SIDS)
  - Children less than 12 months old
  - Most cases cannot be prevent
  - Possible location of the bacteria : inside homes on floors, carpet, and countertops
  - even after cleaning
  - Grow in the intestines and release toxin
  - Feces and serum samples, probable source of the organism

  • **Inhalation Botulism**
  - Rare and not occur naturally (e.g. accidental or intentional such as bioterrorism)
  - scientists have estimated that about 1 gram could potentially kill 1 million people
Symptoms become visible between 1–3 days

- **Intestinal colonization and iatrogenic botulism** - Occur, but rarely
- **Tests for botulinum toxin and for bacteria that cause botulism can be performed at some state health department laboratories and at Centers for Disease Control and Prevention. (CDC)**

4. **Signs and Diagnose of Disease**

- **Signs**: Clear liquids turned milky, cracked jars, loose or dented lids, swollen or dented cans, an “off” order
- **Other diseases with similar symptoms**: stroke, Guillain-Barré syndrome (another disease of muscle paralysis), and myasthenia gravis (causes weakness and eyelid drooping)
- The patient’s history and physical examination may suggest with special tests.
- **Special tests** may need: brain scan, spinal fluid examination, nerve conduction test (electromyography, or EMG), tensilon test for myasthenia gravis
- **Samples** should be obtained for a mouse inoculation test and then the patients should be treated immediately with botulism antiserum.
- **These tests will help distinguish botulism from infections with Salmonella, E. coli, and other Clostridium species (tetanus).**
- **Most direct way** to confirm the diagnosis is to identify the botulinum neurotoxin in the patient's blood, serum, or stool. This is done by injecting the patient's serum or stool into the peritoneal cavity of mice

5. **Symptoms**

- **Symptoms**
  - Double or blurred vision (nearly all patients)
  - Nausea, vomiting, and abdominal cramps but no fever
  - Difficulty swallowing, slurred speech, trouble breathing
  - Fatigue, vertigo
  - Dry mouth, difficulty focusing
  - Neurological symptoms are very
  - Facial weakness on both sides of the face
• **Symptoms of Infant**
  - Constipation (often the first sign)
  - Floppy movements due to muscle weakness
  - Have a weak cry and poor muscle tone
  - Striking loss of head control, paralysis
  - Irritability Drooling
  - Drooping eyelids
  - Difficulty sucking or feeding

6. **Relevant to Environment**

- Funeral of Oregon family wiped out by botulism in 1924. The outbreak was caused by home-canned String beans. Altogether were 12 death. (see pp. 618)
- Translucent pink **Muktuk** (whale skin and blubber), whale meat, and whole Arctic grayling were passed out to guests at the Point Hope Thanksgiving feast. The 40% mortality rate for type E botulism observed among Alaskan natives reflects the difficulty in getting prompt treatment for isolated ethnic group. (see pp. 618)

7. **Treatment and Prevention**

• **Treatment**
  - Antitoxin should be administered as soon as possible after a clinical diagnosis. Early administration is effective in reducing mortality rates.
  - Severe botulism cases require supportive treatment, especially mechanical ventilation, which may be required for weeks or even months.
  - Antibiotics - wound botulism and in infant botulism (secondary infection only)
  - In the U.S. an investigational pentavalent (against neurotoxins A, B, C, D, and E) botulinum toxoid vaccine can be distributed by the CDC for laboratory workers at high risk of exposure to botulinum toxin and by the military for protection of troops against attack. Unfortunately, it takes several months to induce immunity.

**Prevention**

- Boiling the food for 10 minutes over 85°C
- Heating home-canned foods before eat
- Prompt medical care for infected wounds
- Not using injectable street drugs
- Not feed honey for children under 1 years old, till they build up enough acidic juices in their GI tract
- Separate raw and cook
- Keep proper low storage temperature, salt contents and pH (less than pH 4.7)
- Food samples associated with suspect cases must be obtained immediately, stored in properly
- Sealed containers, and sent to laboratories in order to identify the cause and to prevent further cases. (WHO)
- Physicians should immediately report suspected cases of botulism to their state health department. (CDC)

8. Therapeutic and Cosmetic Uses

• Relieving painful muscle contraction in conditions
  - Cerebral palsy, Parkinson disease, multiple sclerosis

• Approved to use
  - To treat severe facial pain caused by trigeminal neuralgia
  - Involuntary eyelid twitching (blepharospasm)
  - Crossed eye (strabismus)
  - Excessive sweating (hyperhidrosis)

• Prevent armpit sweating
  - Twice a year injection
  - Favor by professional models

• Cosmetic
  - Publicized application
  - Periodic local injection to eliminate forehead wrinkle

9. Media to Grow

• C. botulinum can be isolated from food or clinical samples in anaerobic culture.
  Heat or ethanol treatment can aid recovery in highly contaminated samples such as food or feces.

• Mouse bioassay: MBA / the mouse neutralization test (see pp. 619)
  - Only active toxins are detected
- Only approved BoNT detection procedure in the U.S. for clinical samples
- Mice are injected with liquid portion of food extracted or cell-free cultured (e.g. serum, stool, vomitus, gastric content, enema wash with sterile water, food, tissue samples, and culture)
- If the mice die within 72 hours, toxin present.
- To determine the specific type of toxin, groups of mice are passively immunized with antisera specific for C. botulinum type A,B, or E.

**Tryptose sulfite cycloserine (TSC) growth media**
- Isolation and enumeration of vegetative forms as well as spores from Clostridium in food
- C. botulinum cannot metabolize lactose as a primary carbon source
- Must always be incubated in an anaerobic environment with less than 2% of Oxygen
- Requires a pH between 4.8 and 7

**Enzyme-linked Immunosorbent Assays : ELISA**
- Able to detect both active and inactive toxin
- Allows detection of all types of biological molecules at very low concentrations and quantities
- Quick and simple to carry out (results is available in 5 hours)
- Basic immunology concept of an antigen binding to its specific antibody, which allows detection of very small quantities of antigens(proteins, peptides, hormones, or antibody in a fluid sample.
- ELISA utilize enzyme-labeled antigens and antibodies to detect the biological molecules, the most commonly used enzymes being alkaline phosphatase and glucose oxidase.
- Measure the concentration of an analyte (usually antibodies or antigens) in solution
- The steps of the ELISA result in a colored end product which correlates to the amount of analyte present in the original sample
- A chromogenic substrate for the enzyme yields a visible color change or fluorescence, indicating the presence of antigen. Quantitative or qualitative measures can be assessed based on such colorimetric reading.