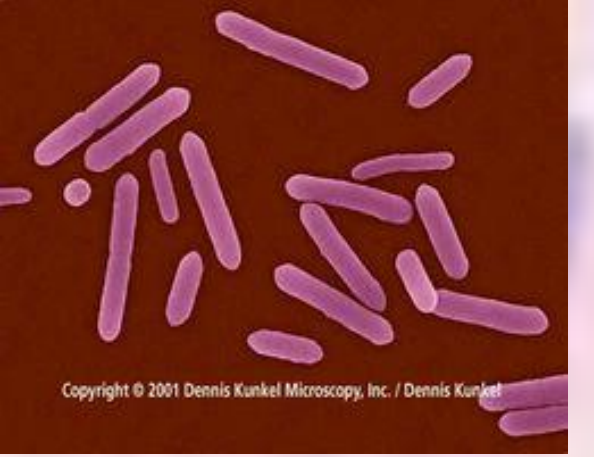
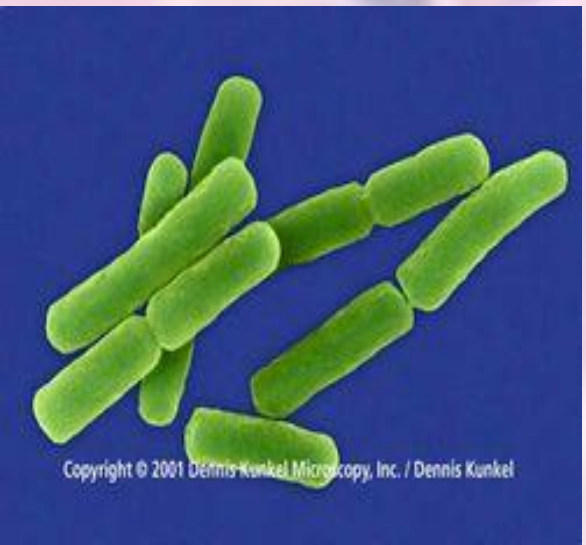
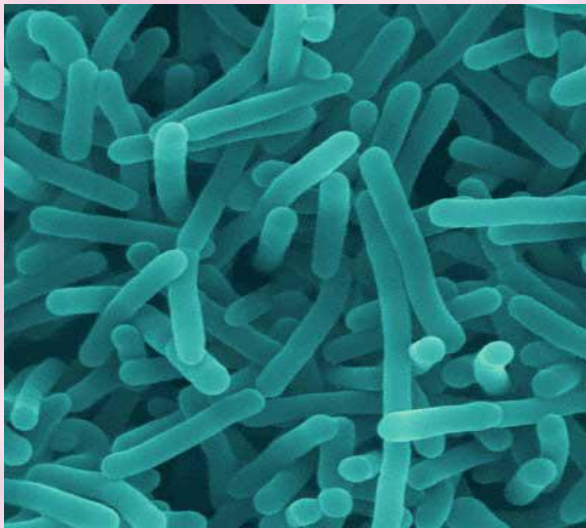


Gram Positive Bacilli

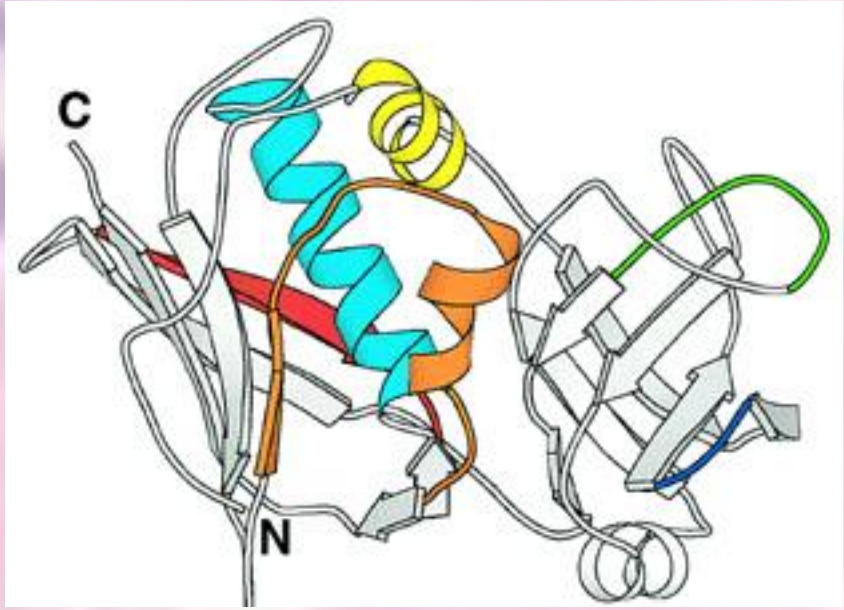




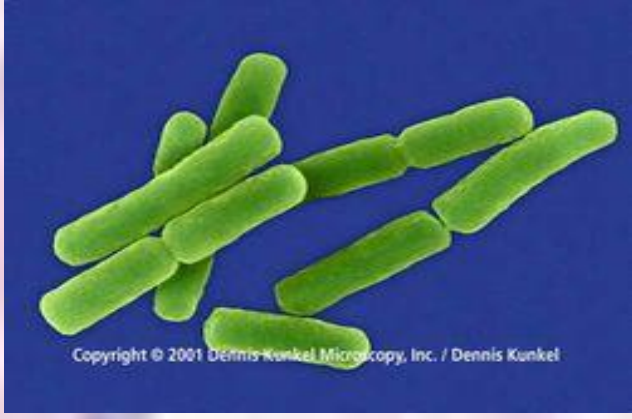
Spore Forming Rods

- *Bacillus* and *Clostridium*

- Release of potent exotoxins causes disease

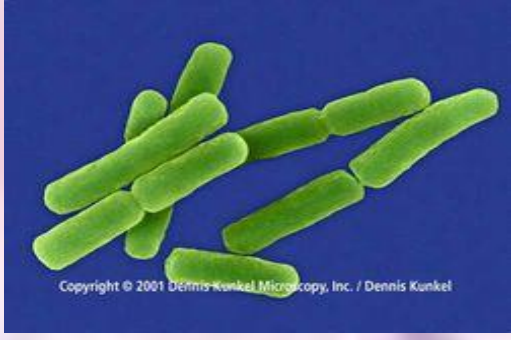


Bacillus anthracis

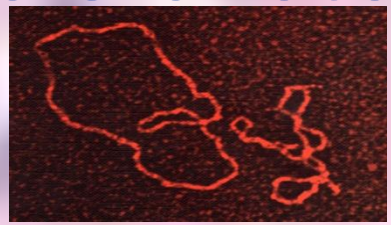


- Causative agent of anthrax
- UNIQUE protein capsule, antiphagocytic
- Aerobic growth conditions
- Spores are very stable, resistant to heat, drying, UV and disinfectants; spores germinate and toxins are made
- Humans exposed to spores usually through contact with animals or soil
- Used in bio-terrorism and warfare

Bacillus anthracis exotoxin



- Encoded on pXO1 plasmid



  - Plasmid contains virulence factors which are transcribed optimally @ 37°C, increased CO_2 and serum proteins

  - ?? Where are these conditions found ??

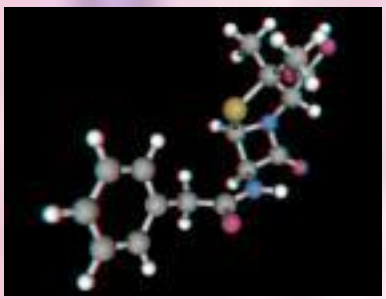
- Exotoxin composed of 3 separate proteins:
  - Edema factor (EF)
  - Protective antigen (PA)
  - Lethal factor (LF)
  - Separately proteins are not toxic, but combined are lethal

- pXO2 plasmid encodes capsule genes

- **BOTH plasmids required for virulence**

Prevention and Treatment

- *RAPID* treatment is essential
- Antibiotics: penicillin, doxycyclin, ciprofloxacin or levofloxacin
- Vaccine against PA protein is available



PENICILLIN

Bacillus cereus

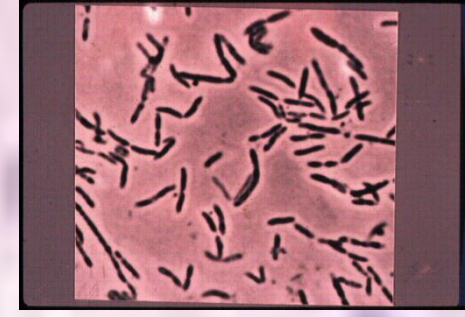
- Causes food poisoning when spores enter food product

- Motile, non-encapsulated, resistant to penicillin

- ENTEROTOXIN is responsible for illness
- 2 types of enterotoxin
  - (i) Heat labile: nausea, abdominal pain, diarrhea. Lasts 12-24hrs
  - (ii) Heat stable: SEVERE nausea and vomiting, short incubation

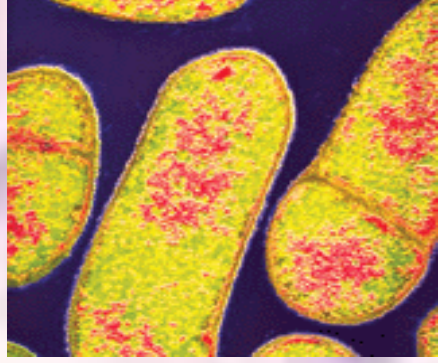
- Antibiotic treatment useless….preformed toxins

Clostridium



- Anaerobic → differentiates this bacteria from other spore-forming bacilli
- Botulism, tetanus, gas gangrene and pseudomembranous colitis
- POWERFUL EXOTOXINS → RAPID DIAGNOSIS

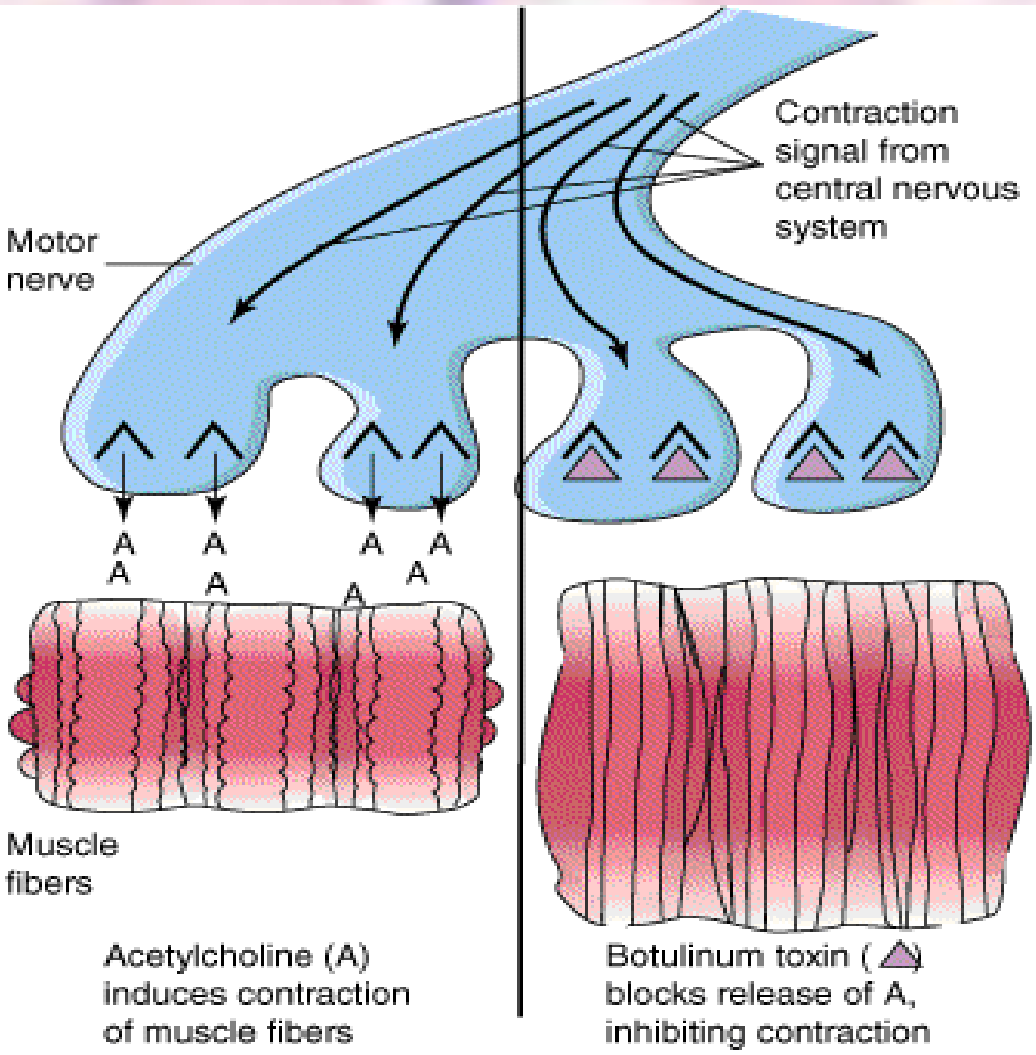
Clostridium botulinum



- Rapidly fatal food poisoning from lethal neurotoxin

- Neurotoxin blocks Ach release in autonomic system; flaccid muscle paralysis:
  - Afebrile, bilateral cranial nerve palsies, double vision, trouble swallowing, muscle weakness
  - Respiratory paralysis → DEATH
  - Treatment: antitoxin and respiratory assistance
- Smoked fish, improperly canned vegetables
  - Proper cooking destroys spores

Action of Botulinum Neurotoxin



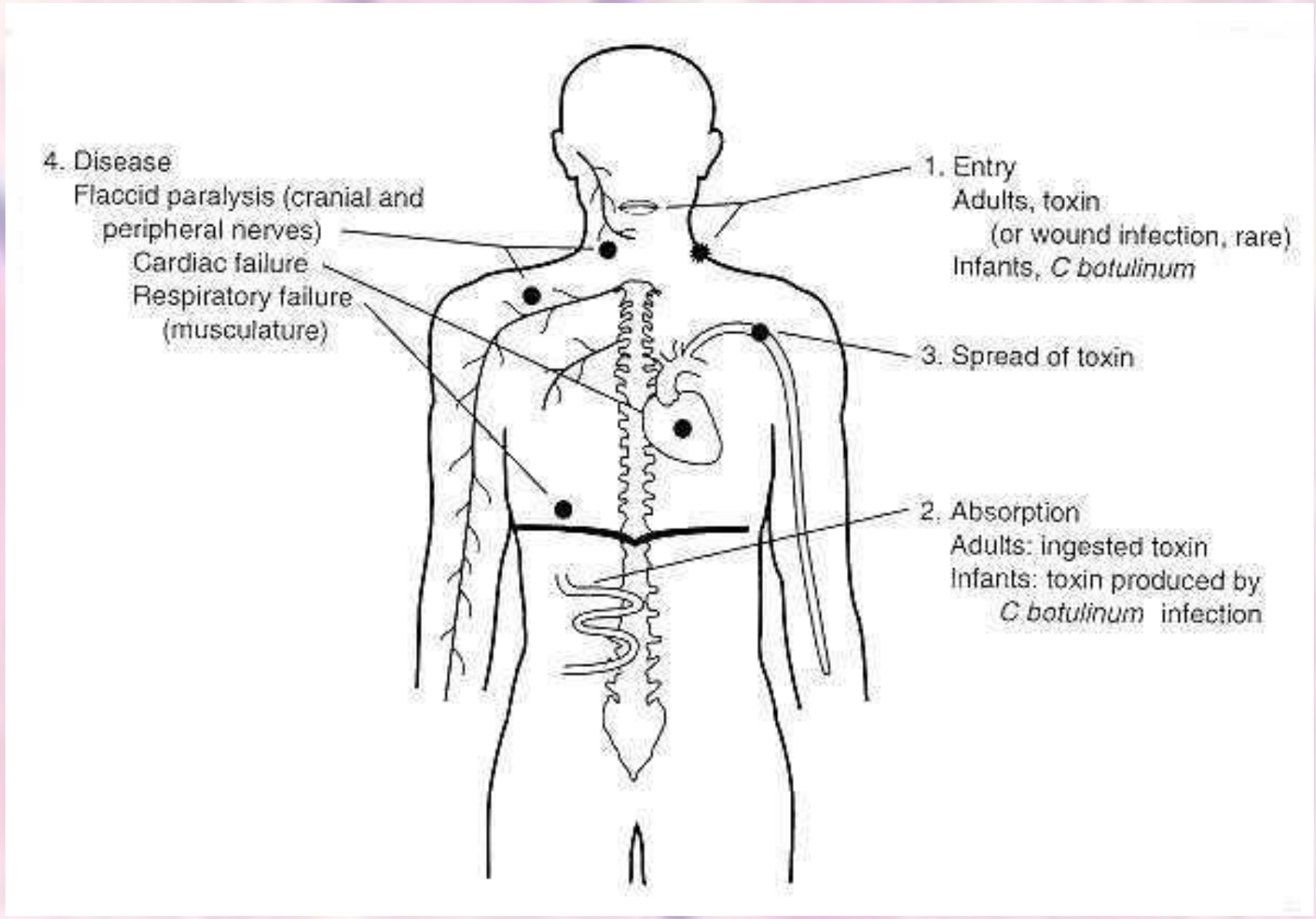
Infant Botulism

- Honey contamination with spores
  - Spores germinate and bacteria colonizes intestine
  - Neurotoxin release

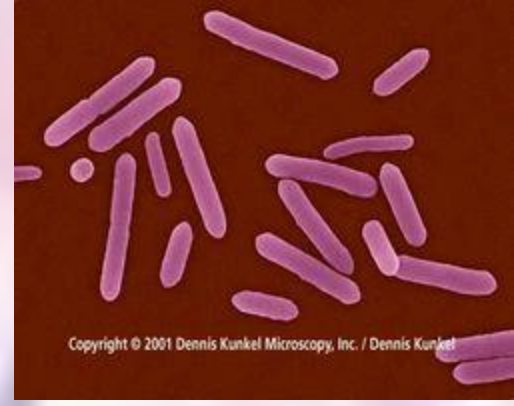
2-3 days of constipation

Trouble swallowing, muscle weakness

Clinical Manifestations of *Clostridium botulinum*



Clostridium tetani

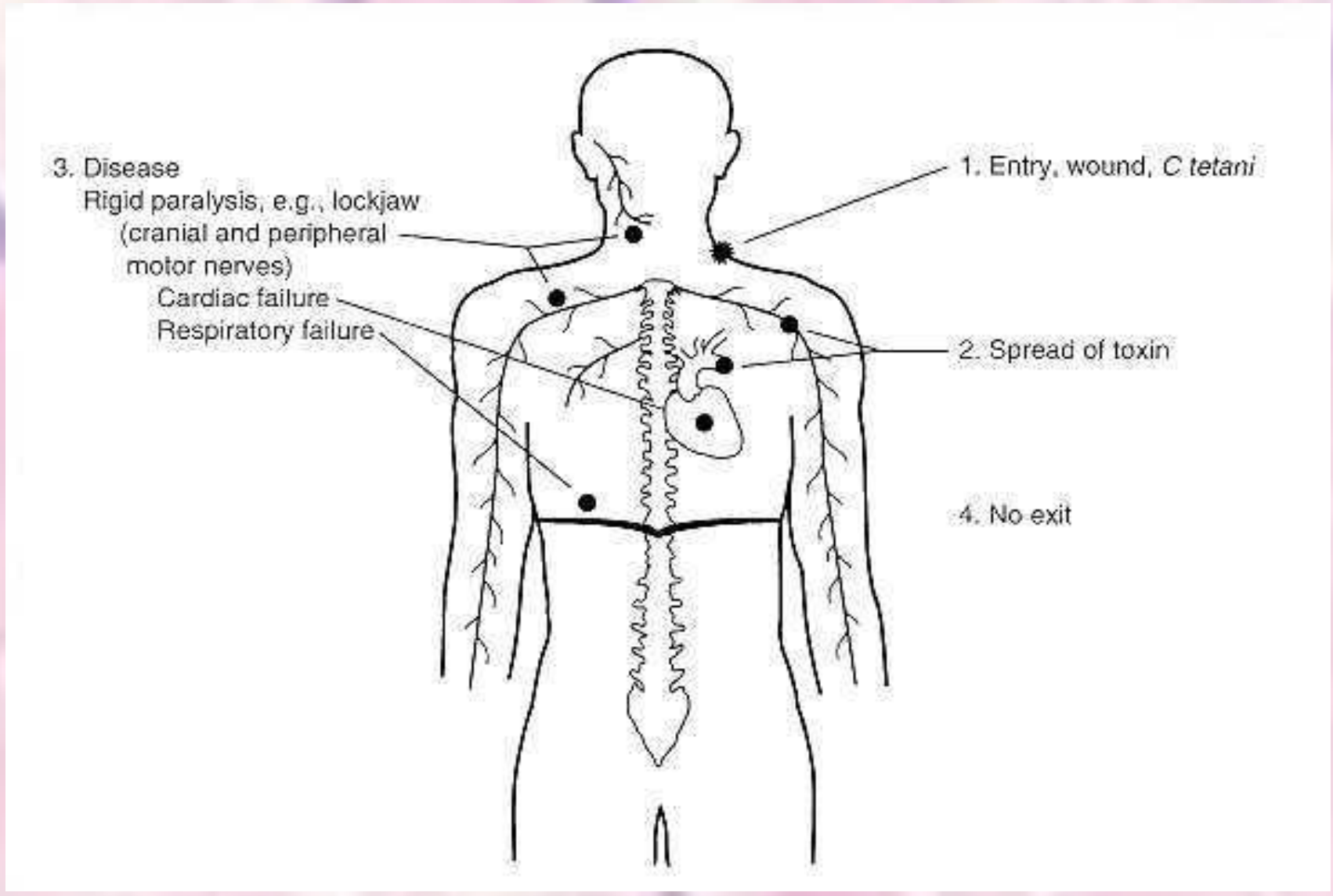


- Tetanus
- Rusty nail contaminated with spores punctures skin; wound provides anaerobic environment

- Exotoxin: tetanospasmin
  - Sustained contraction of skeletal muscles
  - Severe muscle spasms (lock jaw); high mortality at this stage

- Booster (inactivated toxoid) given every 10 years

Clinical manifestations of *Clostridium tetani*



Clostridium perfringens

- Gas gangrene
- Seen in soldiers wounded in battle
- 2 classes of infection:

(i) Wound infection/cellulitis

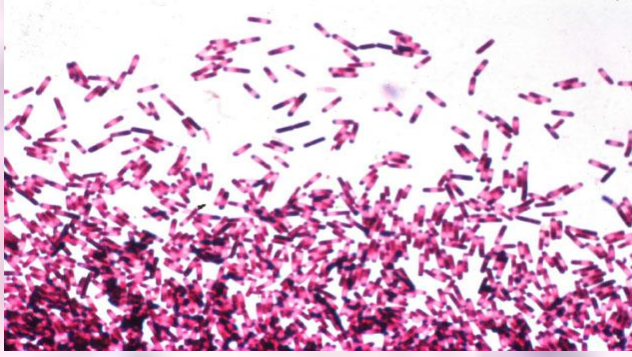
-necrotic skin exposed to bacteria, damage to local tissues; skin feels moist, spongy, with ‘crackly’ pockets

(ii) Clostridial myonecrosis

-bacteria inoculated from trauma into muscles; exotoxin secretion destroys adjacent muscles; as muscles degrade get black fluid excreeted from skin

-FATAL unless treated with oxygen, antibiotics (penicillin) and removal of damaged tissue

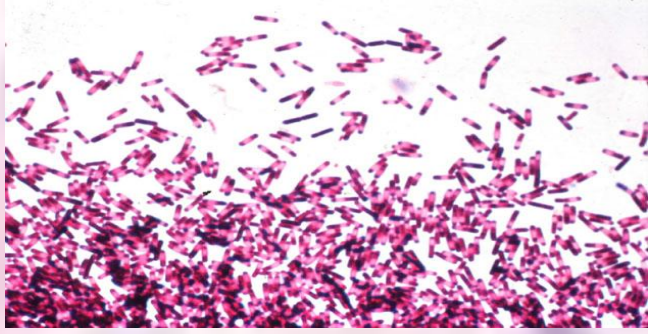
Clostridium difficile



- Causes antibiotic-associated pseudomembranous colitis

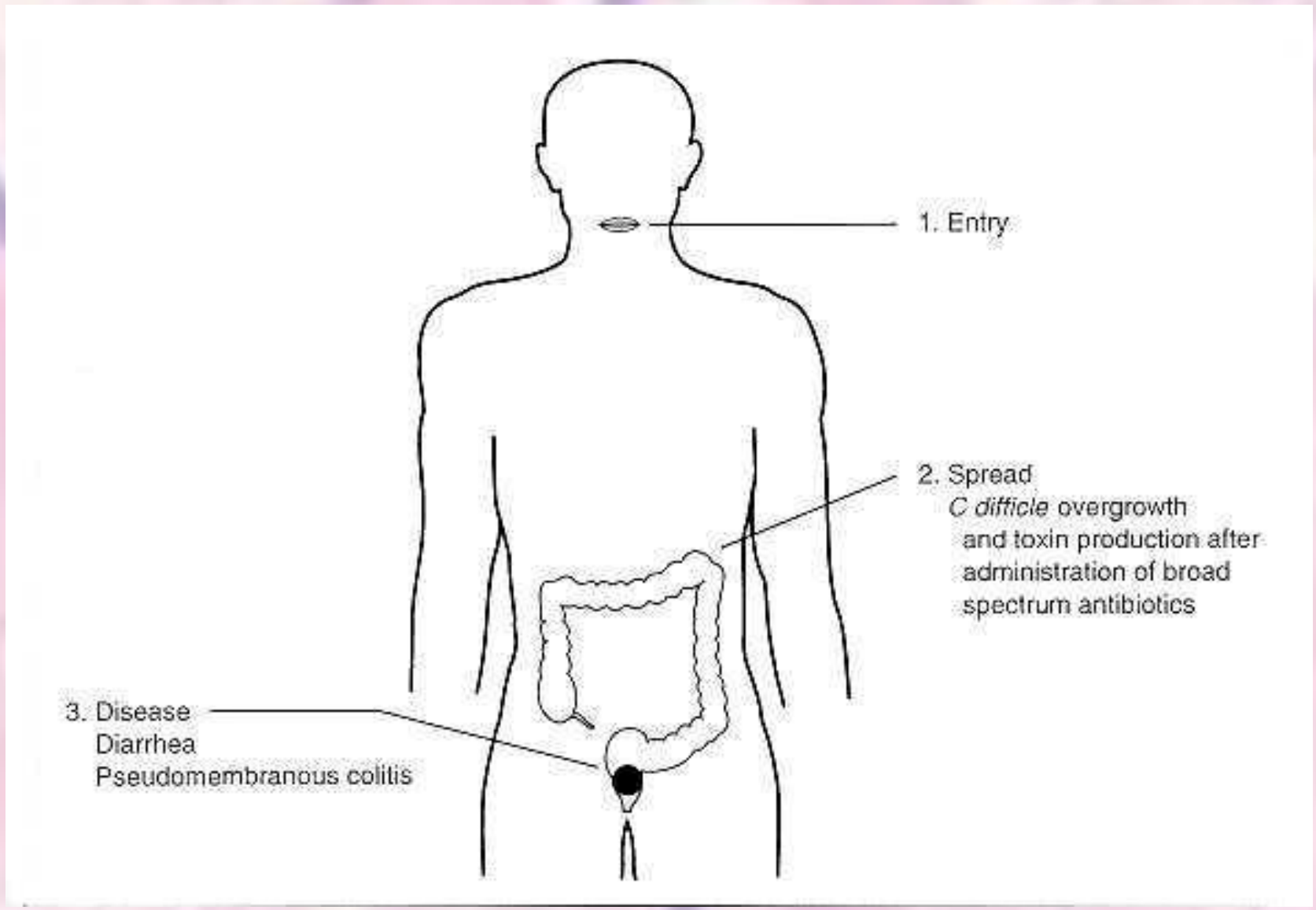
- Seen more commonly in hospitals than tetanus, anthrax or botulism
- Overuse of broad-spectrum antibiotics destroys normal intestinal flora
- Infects colon and releases exotoxins
  - Toxin A: diarrhea
  - Toxin B: cytotoxic to colon cells

Clostridium difficile



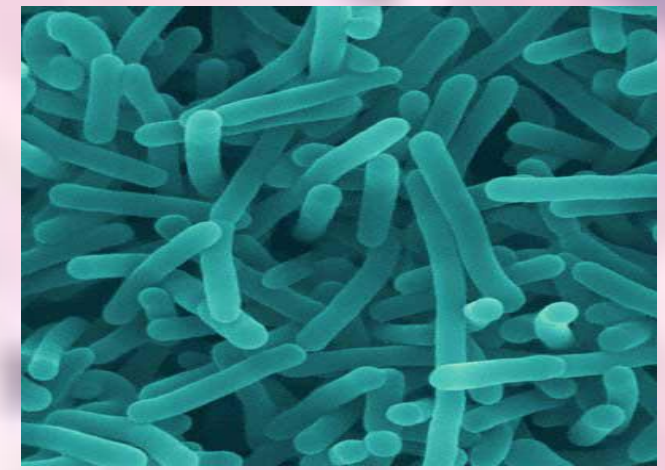
- Symptoms: severe diarrhea, abdominal cramping, fever
- Possible cause of diarrhea in patients on antibiotics
- Treatment
  - discontinue antibiotic treatment
  - Administer metronidazole or vancomycin
    - ?? What is different about these antibiotics ??

Clinical manifestations of *Clostridium difficile*



Non-Spore Forming Rods

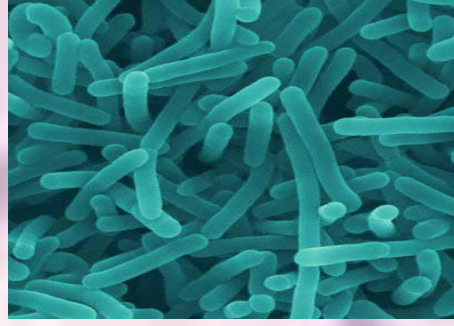
- 2 medically important bacilli
  - *Listeria monocytogenes* and *Corynebacterium diptheriae*



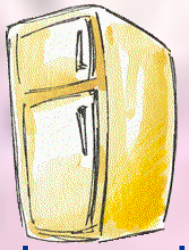
L. monocytogenes

C. diptheriae

Listeria monocytogenes

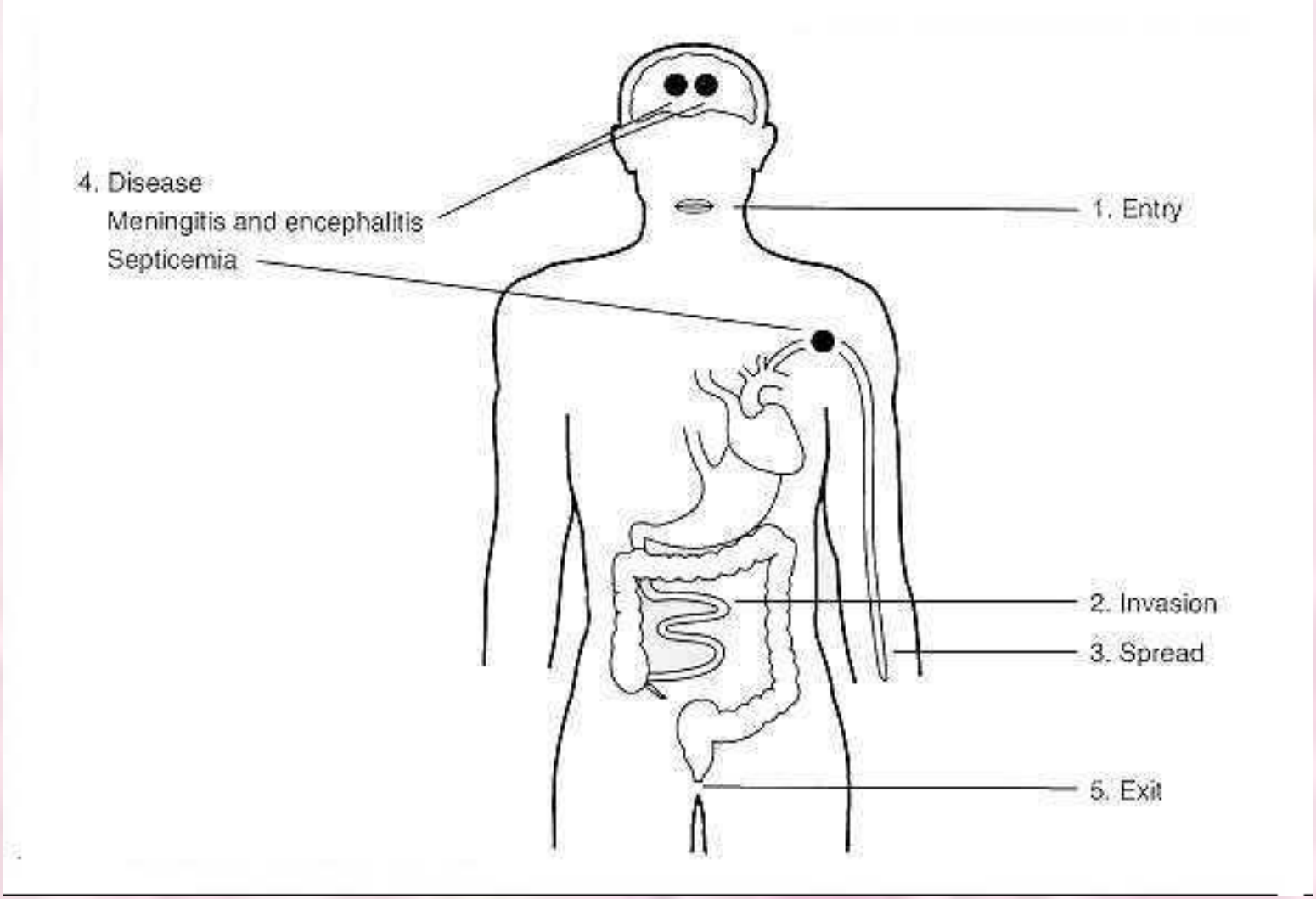


- Causative agent of listeriosis; immunocompromised are at high risk
- Found in foods such as soft cheeses, unpasteurized milk, cold cuts, pâté
- PSYCHROPHILE → survives in refrigerator



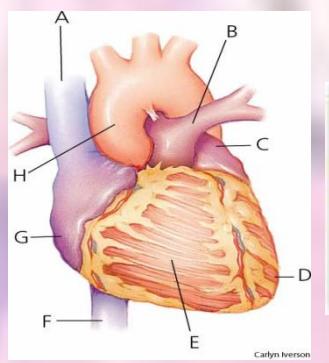
- Variety of symptoms:
  - General malaise, diarrhea, meningitis, septicaemia, still-birth/abortions
- Facultative intracellular aerobe
- Crosses 3 protective barriers (blood-brain, GI and feto-placental)
- Treatment: ampicillin or trimethoprim-sulfamethoxazole

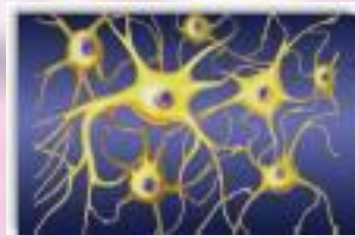
Clinical manifestations of *Listeria monocytogenes*



Corynebacterium diptheriae

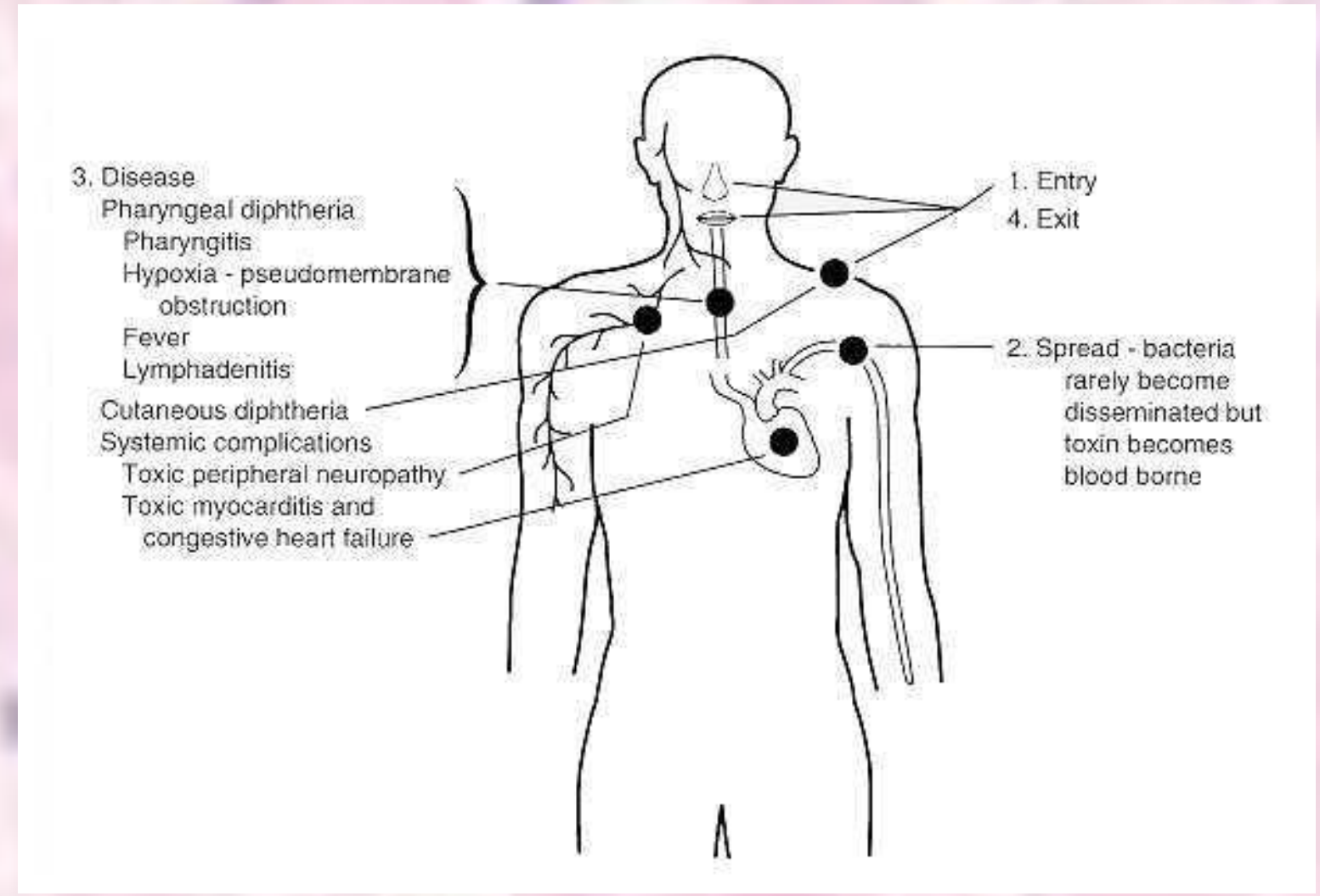
- Causative agent of diptheria
  - Colonization of pharynx and release of exotoxins into bloodstream





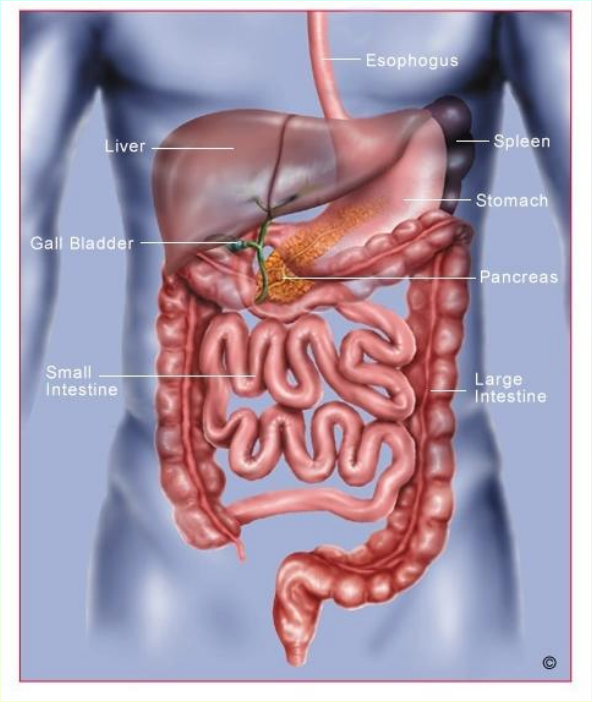
- Exotoxin damages heart and neural cells
- Treatment (3 steps):
  - (i) Antitoxin
  - (ii) Penicillin or erythromycin
  - (iii) DPT vaccine
- Can be lysogenized by a bacteriophage (virus that infects bacteria)

Clinical manifestations of *C. diptheriae*



Gram Negative Bacilli

The Enterics



Enterics

- Found as part of normal intestinal flora BUT can also cause disease
- 4 Major Groups: Enterobacteriaceae (Salmonellae, Shigellae, *E. coli*), Vibrionaceae (*Vibrio, Campylobacter*) Pseudomonadaceae *(Pseudomonas*), Bacterioidaceae
- Organisms are divided into groups based on biochemical and antigenic properties

Biochemical Classifications

- Ability to ferment lactose
  - EMB Media:
    - Lactose fermenters are dark purple/black
    - Inhibits Gram positive bacteria

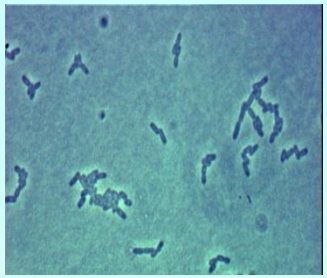
  - MacConkey Media:
    - Lactose fermenters are pink-purple
    - Inhibits Gram positive bacteria

?? What type of media are these ??

SELECTIVE/DIFFERENTIAL

Biochemical Classifications cont'd

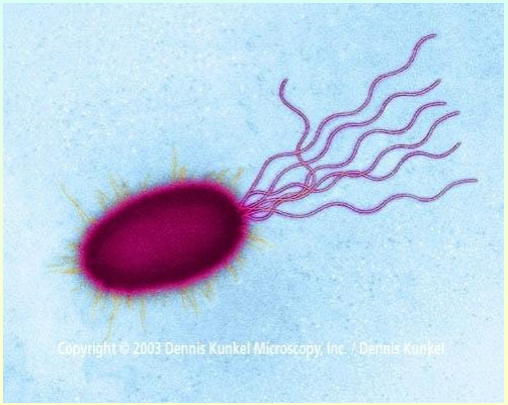
- H_2S production
- Hydrolysis of urea
- Liquefy gelatin



- Decarboxylation of amino acids

Classification Using Surface Antigens

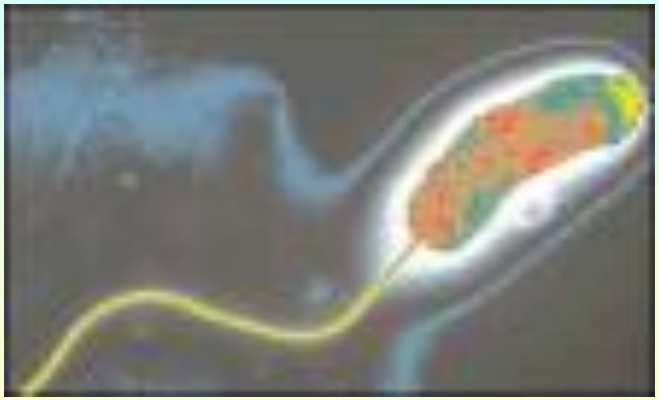
- Variable O-antigen: outermost layer of LPS
    - Changes between enterics
- K-antigen: covers the O-antigen
- H-antigen: flagellar sub-unit
    - Only in motile bacteria



Diseases Caused by Enterics

- Cause diarrhoea with various complications and other infections

1) Diarrhea-with/without systemic invasion
    - Bacteria bind intestinal cells but do not enter
    - EXOTOXIN release causes diarrhoea; ENTEROTOXIN causes fluid/electrolyte loss
    - Watery diarrhoea, NO FEVER
    - *Vibrio cholera*



Vibrio cholera

Enteric Diseases cont'd

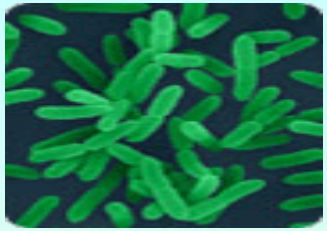
2) Diarrhea with intestinal cell invasion
   - Bacterial virulence factors allow binding and invasion of cells
   - Toxin release destroys cells → bloody stools
   - Fever response
   - *Shigella,*

Enteric Diseases cont'd

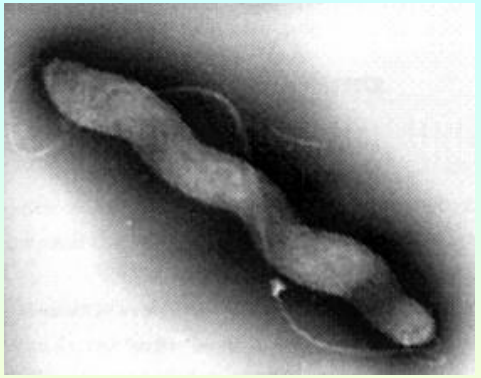
3) Diarrhea with invasion of lymph nodes and bloodstream

- Abdominal pain with diarrhoea containing white and red cells
- Fever, headache, increased white cell counts
- *Salmonella* Typhi, *Yersinia enterocolitica, Campylobacter jejeuni*

S. Typhi



Y. enterocolitica

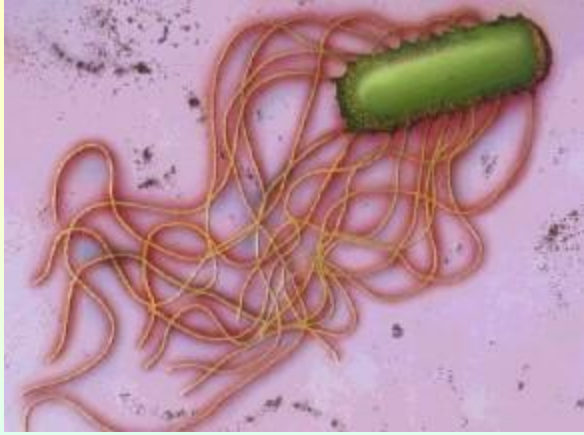


C. jejeuni

Other Enteric Infections

- Urinary tract infects, pneumonia, bacteremia and sepsis
- Nosocomial infections by: *E. coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter, Serratia, Pseudomonas aeruginosa*
- *Pseudomonas aeruginosa*: opportunistic pathogen, often infects burn patients and can disseminate through body into CNS

Salmonellae



- Member of Enterobacteriaceae family
- Unable to ferment lactose
- All have animal reservoirs EXCEPT *S. enterica* serovar Typhi (humans are only host!!)
- Types of infections in humans: enterocolitis, enteric fever, opportunistic infections, septicemia and osteomyelitis

- Two species: *S. enterica* and *S. bongori*

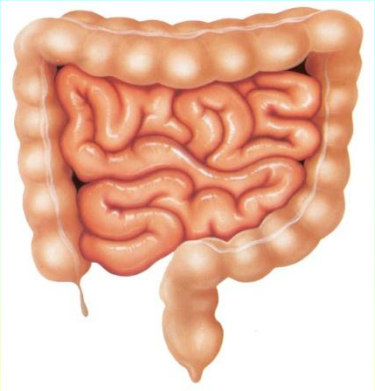
- Enterocolitis (tummy problems):
  - *S. enterica* serovar Enteritidis
  - *S. enterica* serovar Typhimurium

- Enteric fever:
  - *S. enterica* serovar Typhi
  - *S. enterica* serovar Paratyphi

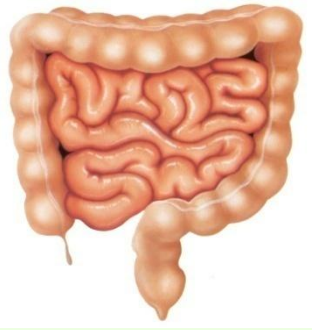
S. enterica serovar Enteritidis

➡ Enterocolitis

- **Pathogenesis depends on:**
  - Dose of ingested organism (min 10^5)
  - Immune status of host
  - Virulence of strain
- Incubation time: 6-48h; multiplication in small intestine



Enterocolitis

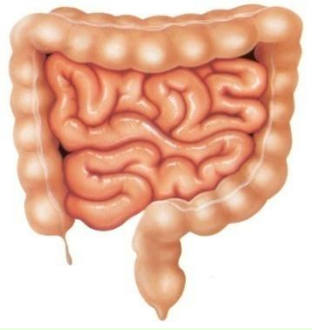


- **Symptoms**
  - Nausea, vomiting, profuse diarrhoea, abdominal pain
  - Fever, chills, headache, myalgia
  - 2-3 days recovery
  - Septicaemia ➡ Rare

- Use stool culture for lab diagnosis

***Salmonella* on MacConkey**

S. enterica serovar Enteritidis ➡ Enterocolitis



- Ingestion of contaminated food
- Poultry, eggs, meat and milk

- Person to person spread
- Most cases occur at home
- Under-reported and undiagnosed
- Antibiotics NOT RECOMMENDED ⟶ WHY?

Enteric Fever

- *S. enterica* serovar Typhy ➡ typhoid fever
- *S. enterica* serotypes Paratyphi A, Schottmuelleri, Hirschfeldii ➡ paratyphoid fever (milder)

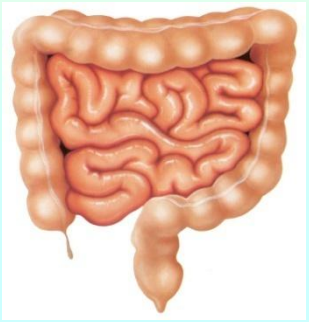
- Enteric fever: generalized infection; bacterial multiplication in lymphoid tissue
- Necrosis of intestinal lymphoid tissue → ulceration, haemorrhage, perforation
- Untreated: 10% mortality
- Convalescent carriers: excrete bacteria for 3 months
- Chronic carriers (1-2%): excrete bacteria for at least 6 months, sometimes life long

Enteric Fever

- Diagnosis: Isolation of bacteria from blood (1st week)
  Stool and urine (2nd-3rd week)
- Infective dose is 10^6 organisms
- Sources are contaminated drinking water, shellfish, milk and milk products
- Clean handling of food, water treatment and safe sewage disposal are essential
- Vaccine available, but only effective against small bacterial load

Escherichia coli

- Most numerous aerobic bacteria of normal gut flora



- Lactose fermenting

***E. Coli* on MacConkey agar**

- Pathogenic to other parts of the body
    - responsible for 85% of bacteriuria

E. coli ➡ Gastroenteritis

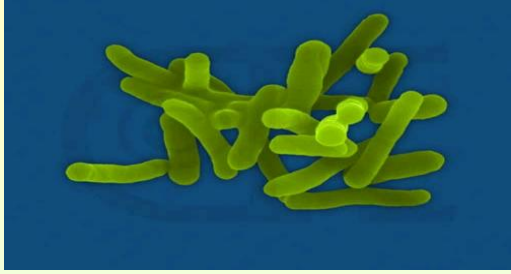
- Enterotoxigenic *E. coli*
  - Infant diarrhoea (developing countries)
  - Traveller's diarrhoea
  - Enterotoxins

- Enteroinvasive *E. coli*
  - Symptoms similar to shigellosis

E. coli ➡ Gastroenteritis

- Enteropathogenic *E. coli*
  - older name for some serotypes causing infant diarrhoea
- *E. coli* O157:H7
  - Haemorrhagic colitis
  - Hamburger disease
  - Proper handling of food, safe preparation and proper cooking practices are essential to prevent illness
- *E. coli* is also implicated in neonatal meningitis and nosocomial urinary and wound infections

Shigellae



- Cause acute diarrhoea with mucus, pus and blood
- Generally non-lactose fermentors
- Dose of 10^5 infects 25% of people, 10^9 infects 95%
- *Shigella sonnei* → Europe and North America

- *S. dysenteriae*:
  - Tropics
  - SEVERE illness: watery diarrhoea, cramps, fever
  - Infection from SMALL numbers of organism
- Most commonly seen in children; poor sanitation and crowding
- Prevention by safe handling of food, treatment of water, safe disposal of sewage
- NO VACCINE

Vibrio cholerae

- Causes cholera: acute gastrointestinal illness
- Profuse watery diarrhoea, cramps and vomiting
- Enterotoxin binds cells in small intestine
    - Cells secrete chlorides, ↓ Na+ absorption
    - Water accumulates in gut → watery diarrhoea
    - Can lead to severe dehydration and death if untreated
- Endemic in South East Asia and parts of Africa
    - Lack of clean drinking water!
- Mainly water-borne
- Massive (10-15 litres per day) loss

Campylobacter

- *C. jejuni* and *C. coli*
- Major cause of human enteritis
- Normal flora in birds and domestic animals

- Some strains invasive, others toxigenic
- Symptoms: fever, abdominal pain, bloody diarrhoea
- Maybe one cause of traveller’s diarrhoea

Pseudomonas

- Opportunistic pathogen
- Found in many moist habitats and water
- Source of infection can be humidifiers etc.
- Treatment is difficult because all *Pseudomonas sp*. are resistant to many antibiotics!!!!

Pseudomonas aeruginosa

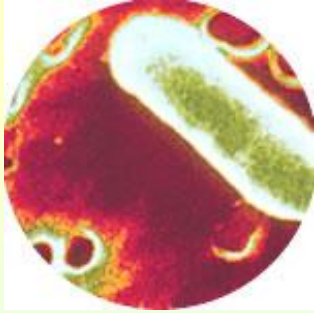
- Respiratory pathogen in cystic fibrosis patients

- Infections in lesions of burn patients

Pseudomonas cepacia

- Common contaminant of saline solutions and water
- Able to multiply in low nutrient environment
- Respiratory pathogen of cystic fibrosis patients

Haemophilus influenzae

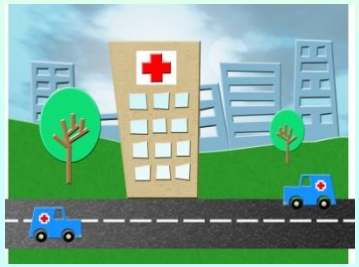


- Part of normal nasopharyngeal flora in many adults and children
- Causes invasive infections of young children

  - Meningitis, pneumonia, joint infections
  - Development of vaccine, now used routinely, decreased the number of cases in Canada
- Can cause increased bronchial inflammation in patients already having chronic bronchitis

Enterobacter spp.

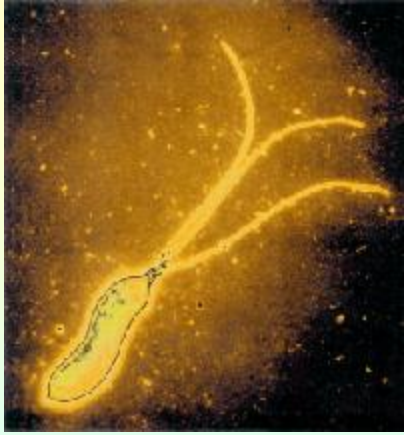
- Can cause nosocomial infections



- Wound infections, pneumonia, bacteremia
- *E. sakazakii* linked to infant illness from powdered infant formula

Now called *Cronobacter*

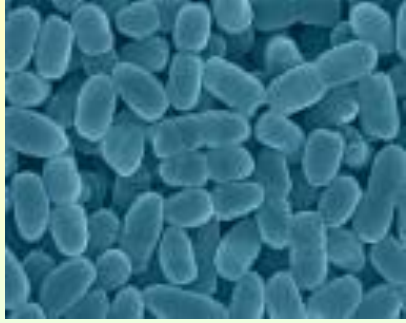
Helicobacter pylori



- Microaerophillic, spiral bacilli
- Most common cause of stomach ulcers
    - In the past, cause was thought to be stress and diet
    - 1982-Dr.Robin Warren and Dr. Barry Marshall discovered link between *H. pylori* and ulcers
    - Medical community slow to accept their theory (1994-National Institute of Health Conference concludes strong association between ulcers and *H. pylori*)
- Urease: protection form low pH
- Triple therapy treatment: antibiotics and H+ pump inhibitors

Duodenal Ulcer

Bordetella pertussis



- Whooping cough *VIOLENT COUGH*
- 4 Virulence Factors
  - Pertussis toxin (A-B)
  - Extra cytoplasmic adenylate cyclase (weakens host defense)
  - Filamentous hemagglutinin (bronchial attachment and exotoxoin release)
  - Tracheal cytotoxin (destroys ciliated cells → poor clearance of mucous and bacteria)
- Prevention: vaccination with heat-killed organism

Legionella pneumophila

- Causes Legionnaires disease
- Opportunistic pathogen
- May cause severe pneumonia
- Grows in water and is found in shower heads, water tanks, air cooling/heating tanks
- Exposure is by aerosol and there is NO person-to-person transmission