Clostridium

See Lecture Handouts

Clostridium spp. Anaerobic Gram-Positive Spore-Forming Bacilli

Four broad types of pathogenesis:

1. **Histotoxic group — tissue infections**

(***C. perfringens* type A**, exogenously acquired more commonly than endogenously)
(**C. *septicum***; endogenously-acquired)

a. cellulilitis
b. myonecrosis
c. gas gangrene
d. fasciitis

2. **Enterotoxigenic group — gastrointestinal disease**

a. clostridial foodbome disease (8-24h after ingestion of large numbers of organisms on con-taminated meat products, spores germinate, enterotoxin produced (***C. perfringens* type A**)
b. necrotizing enteritis (beta toxin-producing ***C.perfringens* type C**)

(***C. difficile*** endogenously-acquired or exogenously-acquired person-to-person in hospital)
c. antibiotic-associated diarrhea
d. antibiotic-associated pseudomembrane colitis

3. **Tetanus** (exogenously acquired) — C. *tetani* neurotoxin

a. generalized (most common)
b. cephalic(primary infection in head, comnnonly ear)
c. localized
e. neonatal (contaminated umbilical stump)

4. **Botulism** (exogenously acquired) — *C. botulinum* neurotoxin

a. foodborne (intoxication, 1-2days incubation period)
b. infant (ingestion of spores in honey)
c. wound (symptoms similar to foodborne, but 4 or more days incubation)

Spores

- *Clostridium* form endospores under **adverse environmental conditions**
- Spores are a **survival** mechanism
- Spores are characterized on the basis of position, size and shape
- Most *Clostridium* spp., including ***C. perfringens*** **and *C. botulinum*, have ovoid subterminal (OST) spores**
- ***C. tetani*** **have round terminal (RT) spores**

Clostridium Associated Human Disease

Species	Human Disease	Frequency
C. difficile	Antibiotic-associated diarrhea, pseudomembranous colitis	Common
C. perfringens	Soft tissue infections (i.e., cellulitis, suppurative myositis, myonecrosis or gas gangrene), food poisoning, enteritis necroticans, septicemia	Common
C. septicum	Gas gangrene, septicemia	Uncommon
C. tertium	Opportunistic infections	Uncommon
C. botulinum	Botulism	Uncommon
C. tetani	Tetanus	Uncommon
C. barati	Botulism	Rare
C. butyricum	Botulism	Rare
C. histolyticum	Gas gangrene	Rare
C. novyi	Gas gangrene	Rare
C. sordellii	Gas gangrene	Rare

Clostridium perfringens

Clostridium perfringens — histotoxic or enterotoxigenic infections

Morphology and Physiology

- **large, rectangular bacilli** (rod) staining **gram-positive**
- **spores rarely seen** *in vitro* or in clinical specimens (ovoid, subterminal)
- **non-motile,** but **rapid spreading growth** on blood agar mimics growth of motile organisms
- aerotolerant, especially on media supplemented with blood
- grow at temperature of 20-50°C (optimum 45°C) and pH of 5.5-8.0

Pathogenicity Determinants (note that toxins include both cytolytic enzymes and bipartite exotoxins)

- four **major lethal toxins** (alpha (α), beta (β), epsilon (ε), and iota (ι) toxins) and an **enterotoxin**
- **six minor toxins** (delta(δ), theta(θ), kappa(κ), lambda(λ), mu(μ), nu(η)toxins) **& neuraminadase**
- C. *perfringens* subdivided into five types (A-E) on basis of production of major lethal toxins
- **C. *perfringens* Type A** (only major lethal toxin is alpha toxin) responsible for histotoxic and enterotoxigenic infections in humans; Type C causes necrotizing enteritis (not in U.S.)

Lab Identification

- direct smear and Gram stain, capsules upon direct examination of wound smears
- culture takes advantage of **rapid growth** in chopped meat media at 45° C to enrich and then isolate onto blood agar streak plate after four to six hours
- **gas** from glucose fermentation
- in vivo toxicity testing and identification of the specific toxin types involved
- **double zone of hemolysis** on blood agar (p-hemolytic theta(e) toxin, a-hemolytic alpha(oc) toxin)
- **Nagler rxn;** precipitation in serum or egg yolk media; oc -toxin (phospholipase C) is a lecithinase
- **"stormy" fermentation** (coagulaltion) of milk due to large amounts of acid and gas from lactose

Diagnosis/Treatment of systemic infection — Early diagnosis and aggressive treatment essential

- removal of necrotic tissue **(surgical debridement)**
- **Penicillin G in high doses** if more serious infection

  Of poorly defined clinical value are:
  - administration of antitoxin
  - hyperbaric oxygen (dive chamber) adjunct therapy (??inhibit growth of anaerobe??)

Summary of C. perfringens Infections

Physiology and Structure

Large, rectangular, gram-positive bacillus.

Forms spores but they are rarely seen in clinical specimens or culture.

Replicates rapidly, so large spreading colonies are seen within first day of culture; "double zone" of hemolysis on blood agar (due to α and δ toxins).

Produces many toxins and hemolytic enzymes, so white blood cells are not seen in Gram-stained clinical specimens.

Produces lecithinase (phospholipase C).

Subdivided into 5 types (A–E) on the basis of toxin production (refer to Table 37–2).

Virulence

Refer to Table 37–3.

Epidemiology

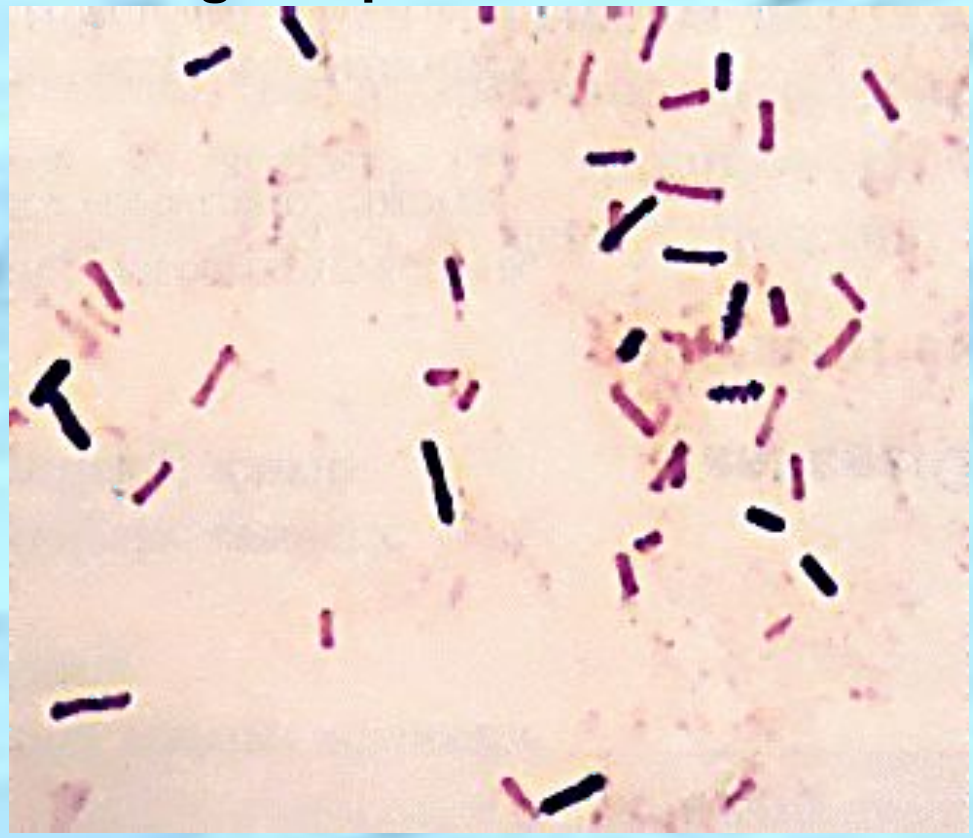
Ubiquitous; present in soil, water, and intestinal tract of humans and animals.

Type A is responsible for most human infections (also only type capable of surviving in soil).

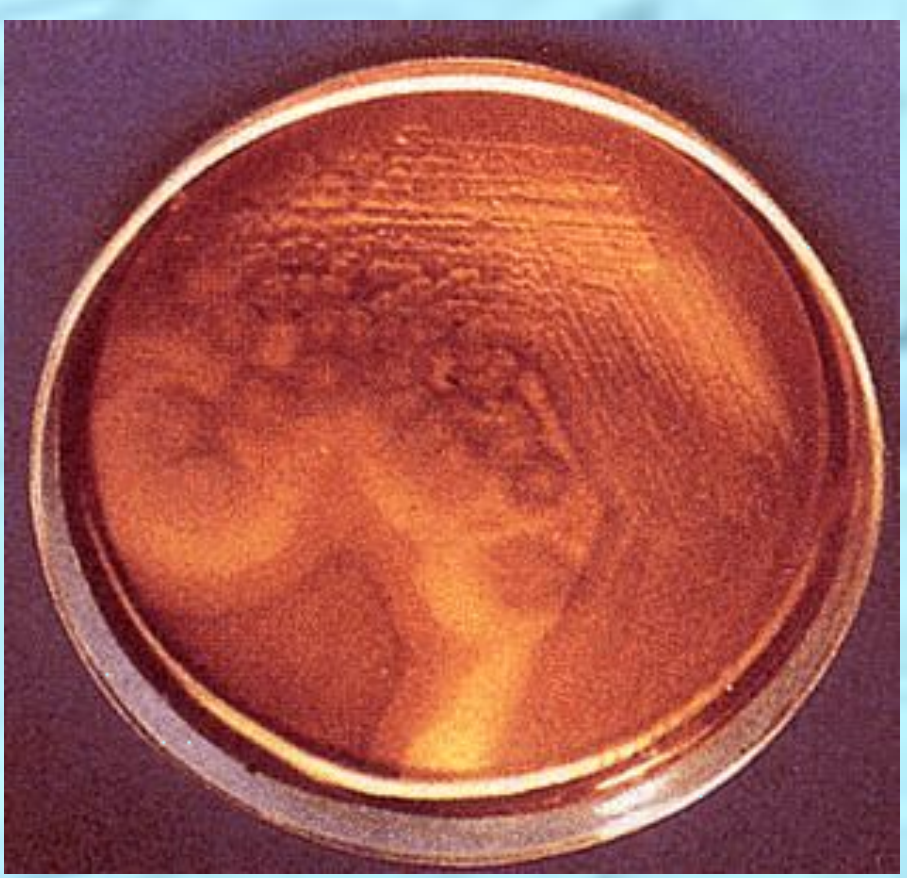
Disease follows exogenous or endogenous exposure.

Micro & Macroscopic C. perfringens

NOTE: Large rectangular gram-positive bacilli



NOTE: Double zone of hemolysis



Inner beta-hemolysis = θ toxin
Outer alpha-hemolysis = α toxin

Summary of C. perfringens Infections (cont.)

Diseases

Soft tissue infections (cellulitis, suppurative myositis, myonecrosis).

Food poisoning.

Septicemia.

Diagnosis

Characteristic forms seen on Gram stain.

Grows rapidly in culture.

Treatment, Prevention, and Control

Rapid treatment is essential for serious infections.

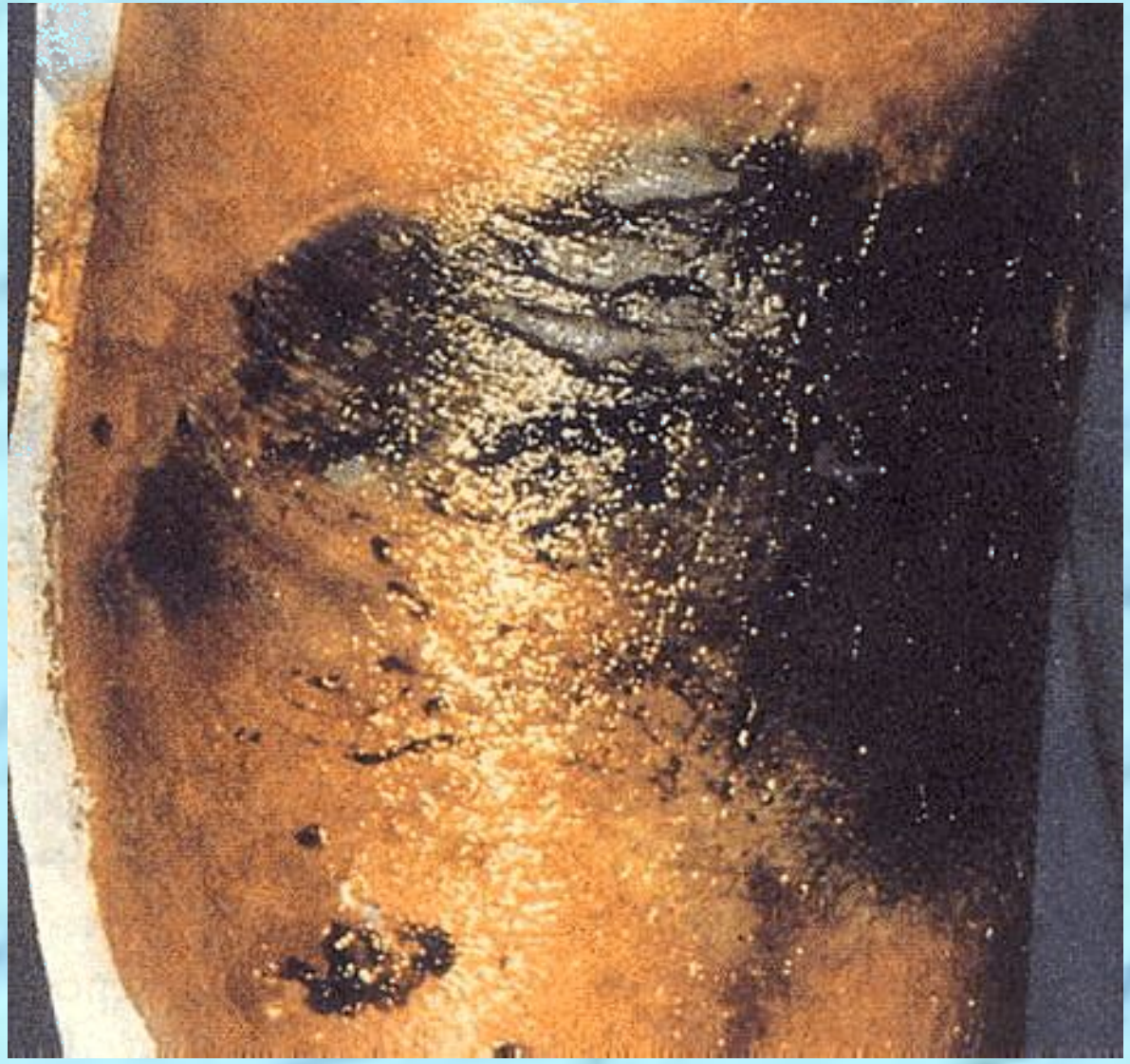
Systemic infections require surgical débridement and high-dose penicillin therapy; antiserum against α toxin not used now, and the value of hyperbaric oxygen treatment is unproven.

Treat with débridement and penicillin for localized infections.

Symptomatic treatment for food poisoning.

Proper wound care and judicious use of prophylactic antibiotics will prevent most infections.

Clostridial Cellulitis



C. perfringens Virulence Factors

	Virulence Factors	Biologic Activity
Major	α toxin	Lethal toxin; phospholipase C (lecithinase); increases vascular permeability; hemolysin; produces necrotizing activity
Major	β toxin	Lethal toxin; necrotizing activity
Major	ϵ toxin	Lethal toxin; permease
Major	ι toxin	Lethal binary toxin; necrotizing activity; adenosine diphosphate (ADP) ribosylating
Minor	δ toxin	Hemolysin
Minor	θ toxin	Heat- and oxygen-labile hemolysin; cytolytic
Minor	κ toxin	Collagenase; gelatinase; necrotizing activity
Minor	λ toxin	Protease
Minor	μ toxin	Hyaluronidase
Minor	ν toxin	Deoxyribonuclease; hemolysin; necrotizing activity
	Enterotoxin	Alters membrane permeability (cytotoxic, enterotoxic)
	Neuraminidase	Alters cell surface ganglioside receptors; promotes capillary thrombosis

Exotoxins Associated with C. perfringens Types A-E

Type of Isolate	Major Lethal Toxins			
	α	β	ϵ	ι
A	+	−	−	−
B	+	+	+	−
C	+	+	−	−
D	+	−	+	−
E	+	−	−	+

C. perfringens Nagler Reaction

NOTE: **Lecithinase (α-toxin; phospholipase) hydrolyzes phospholipids in egg-yolk agar around streak on right. Antibody against α-toxin inhibits activity around left streak.**

Clostridium tetani

Clostridium tetani — agent of tetanus

Morphology and Physiology-

- long thin gram-positive organism that stains gram negative in old cultures
- **round terminal spore** gives drumstick appearance
- motile by peritrichous flagella
- grow on blood agar or cooked meat medium with swarming
- beta-hemolysis exhibited by isolated colonies
- spores resist boiling for 20 minutes

Antigenic Structure-

flagella (H), somatic (0), and spore antigens. Single antigenic toxin characterizes all strains.

Pathogenicity Determinants"

- play a role in local infection only in conjunction with other bacteria that create suitable environment for their invasion
- systemic-acting, **plasmid-mediated A-B neurotoxin (tetanospasmin)** produced intracellularly
  - Mode of Action — one of most poisonous substances
    - binds gangliosides in synaptic membranes (synapses of neuronal cells) and **blocks release of inhibitory neurotransmitters;** continuous stimulation by excitatory transmitters
    - muscle spasms (spastic paralysis) (trismus (lockjaw), risus sardonicus, opisthotonos), cardiac arrhythmias, fluctuations in blood pressure

Lab Identification"

- use characteristics of resistance to heat, motility, and toxin production to help identify

Diagnosis/Treatment/Prevention

- empirical diagnosis on basis of clinical manifestations
- treat to prevent elaboration and absorption of toxin
  - **clean wound** (debridement), **control spasms**
  - **metronidazole** administered to eliminate vegetative bacteria that produce neurotoxin
  - **passive immunity** (human tetanus immunoglobulin); **vaccination** (active) as preventative
  - **antitoxin** administered to bind free tetanospasmin

Summary of C. tetani Infections

Physiology and Structure

Gram-positive bacilli with prominent terminal spores (drumstick appearance).

Strict anaerobe (vegetative cells are extremely oxygen sensitive).

Difficult to isolate from clinical specimens.

Virulence

Spore formation.

Tetanospasmin (heat-labile neurotoxin; blocks release of neurotransmitters [i.e., gamma-aminobutyric acid, glycine] for inhibitory synapses).

Tetanolysin (heat-stable hemolysin of unknown significance).

Epidemiology

Ubiquitous; spores are found in most soils and can colonize gastrointestinal tract of humans and animals.

Exposure to spores is common, but disease is uncommon except in underdeveloped countries, where there is poor vaccination compliance and medical care is inadequate.

Risk is greatest for people with inadequate vaccine-induced immunity; disease does not induce immunity.

Summary of Clostridium tetani Infections (cont.)

Diseases

Generalized tetanus (most common form).

Cephalic tetanus (high mortality).

Localized or wound tetanus (good prognosis).

Neonatal tetanus (high mortality).

Diagnosis

Diagnosis is based on clinical presentation.

Microscopy and culture with poor sensitivity.

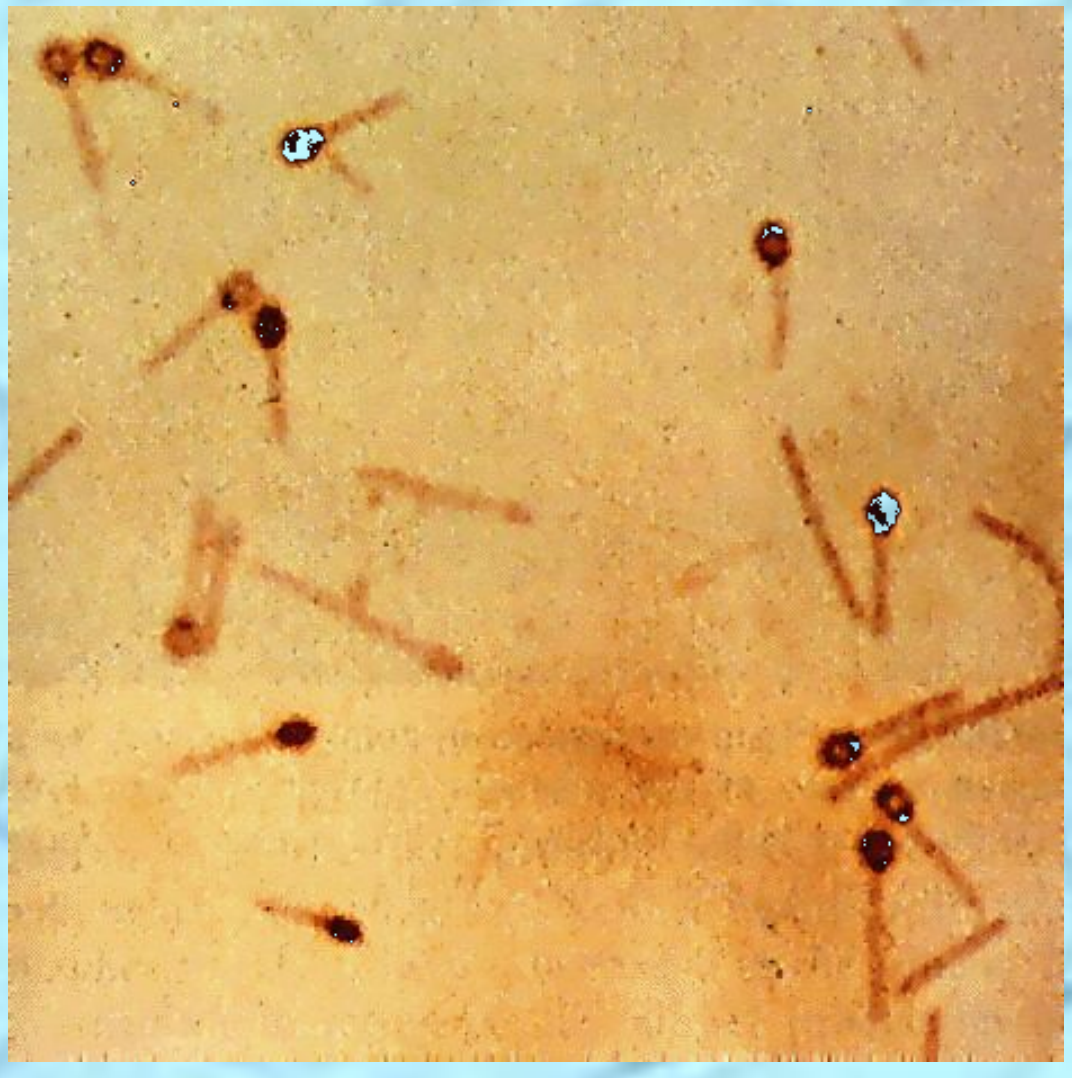
Neither tetanus toxin nor antibodies are typically detected.

Treatment, Prevention, and Control

Treatment requires débridement, antibiotic therapy (metronidazole), passive immunization with antitoxin globulin, and vaccination with tetanus toxoid.

Prevention through use of vaccination, consisting of three doses of tetanus toxoid followed by boosters every 10 years.

Clostridium tetani Gram Stain

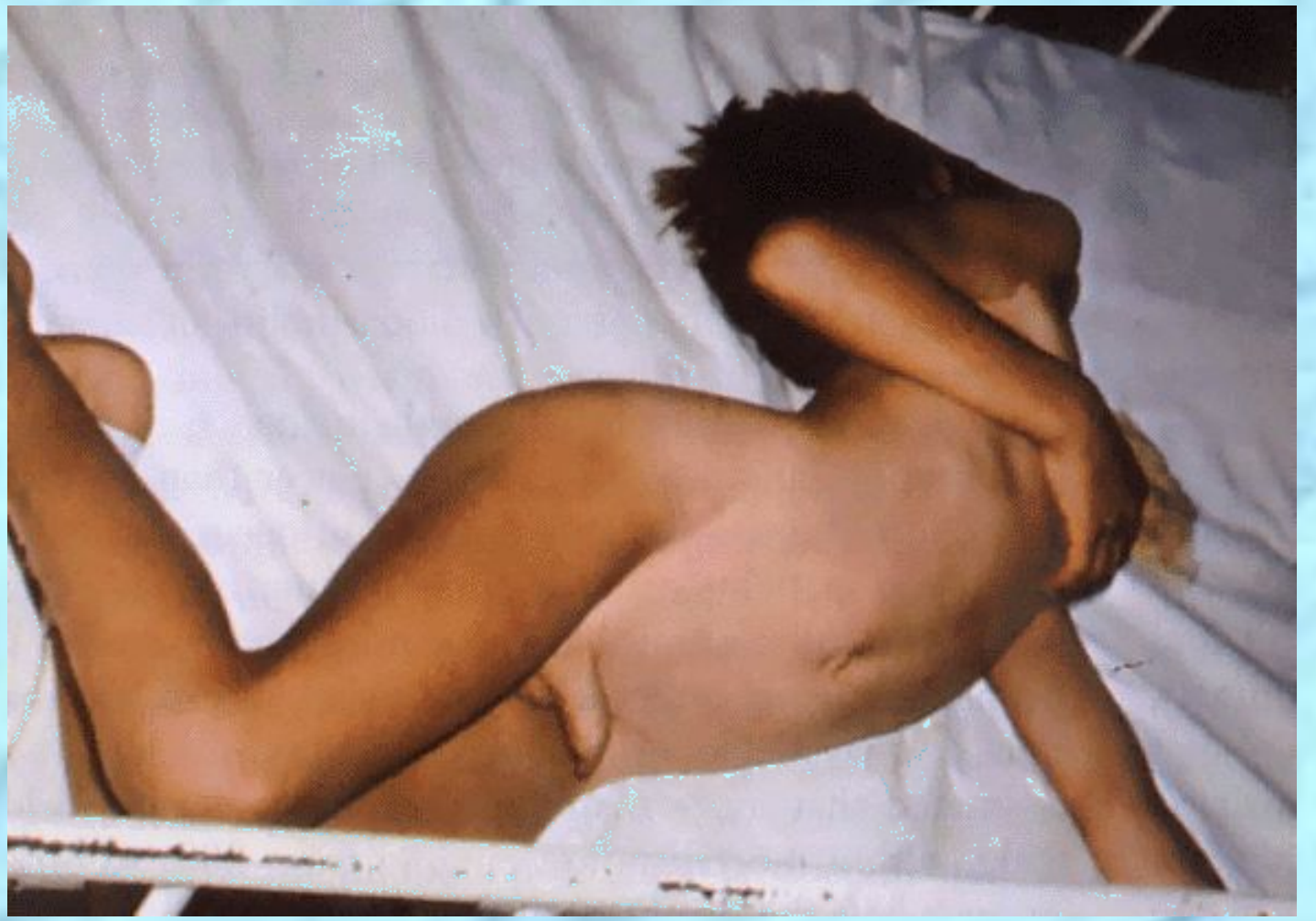


NOTE: **Round terminal spores give cells a "drumstick" or "tennis racket" appearance.**

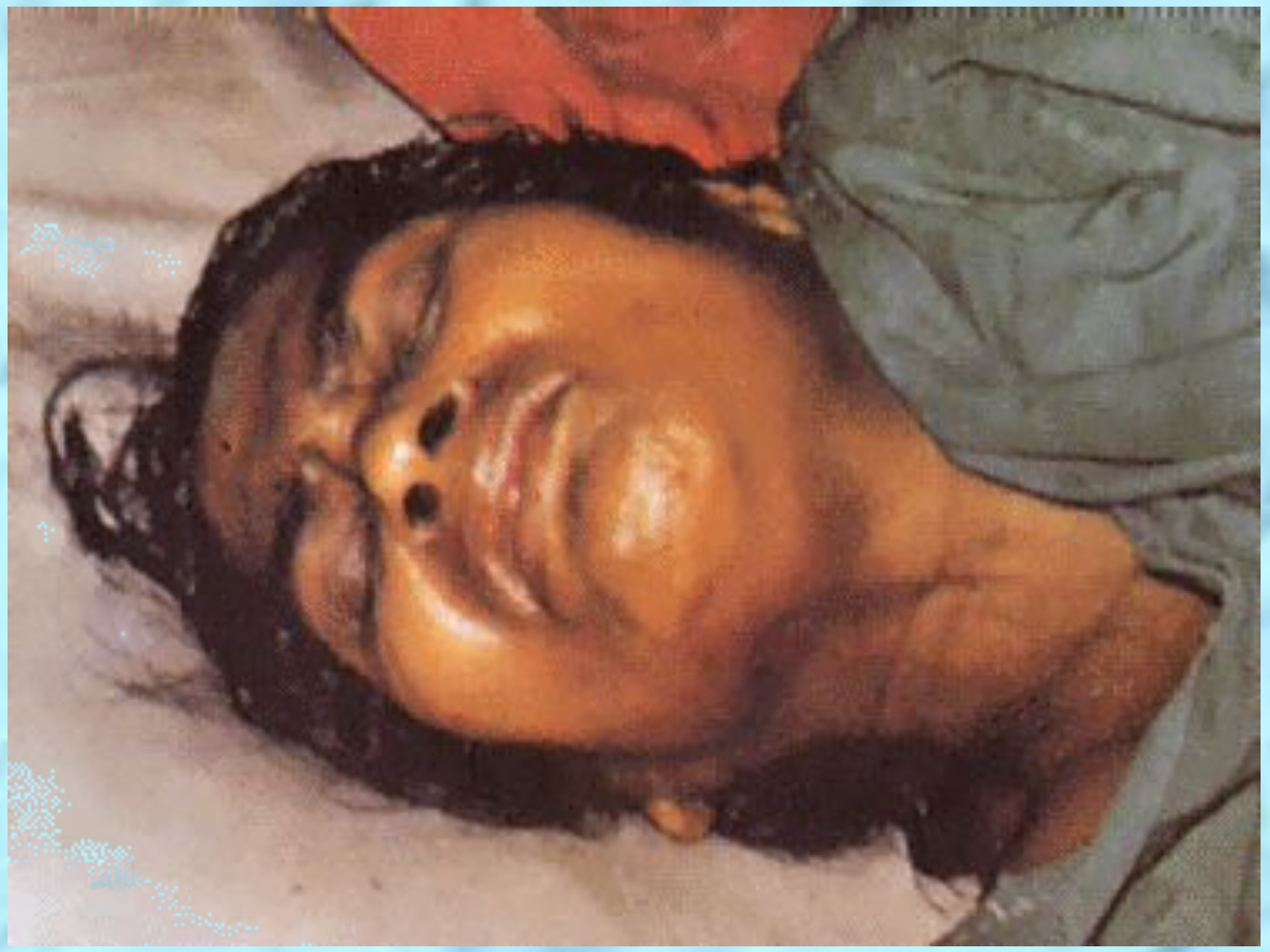
Clinical Forms of Tetanus

Disease	Clinical Manifestations
Generalized	Involvement of bulbar and paraspinal muscles (trismus or lockjaw, risus sardonicus, difficulty swallowing, irritability, opisthotonos); involvement of autonomic nervous system (sweating, hyperthermia, cardiac arrhythmias, fluctuations in blood pressure)
Cephalic	Primary infection in head, particularly ear; isolated or combined involvement of cranial nerves, particularly seventh cranial nerve; very poor prognosis
Localized	Involvement of muscles in area of primary injury; infection may precede generalized disease; favorable prognosis
Neonatal	Generalized disease in neonates; infection typically originates from umbilical stump; very poor prognosis in infants whose mothers are nonimmune

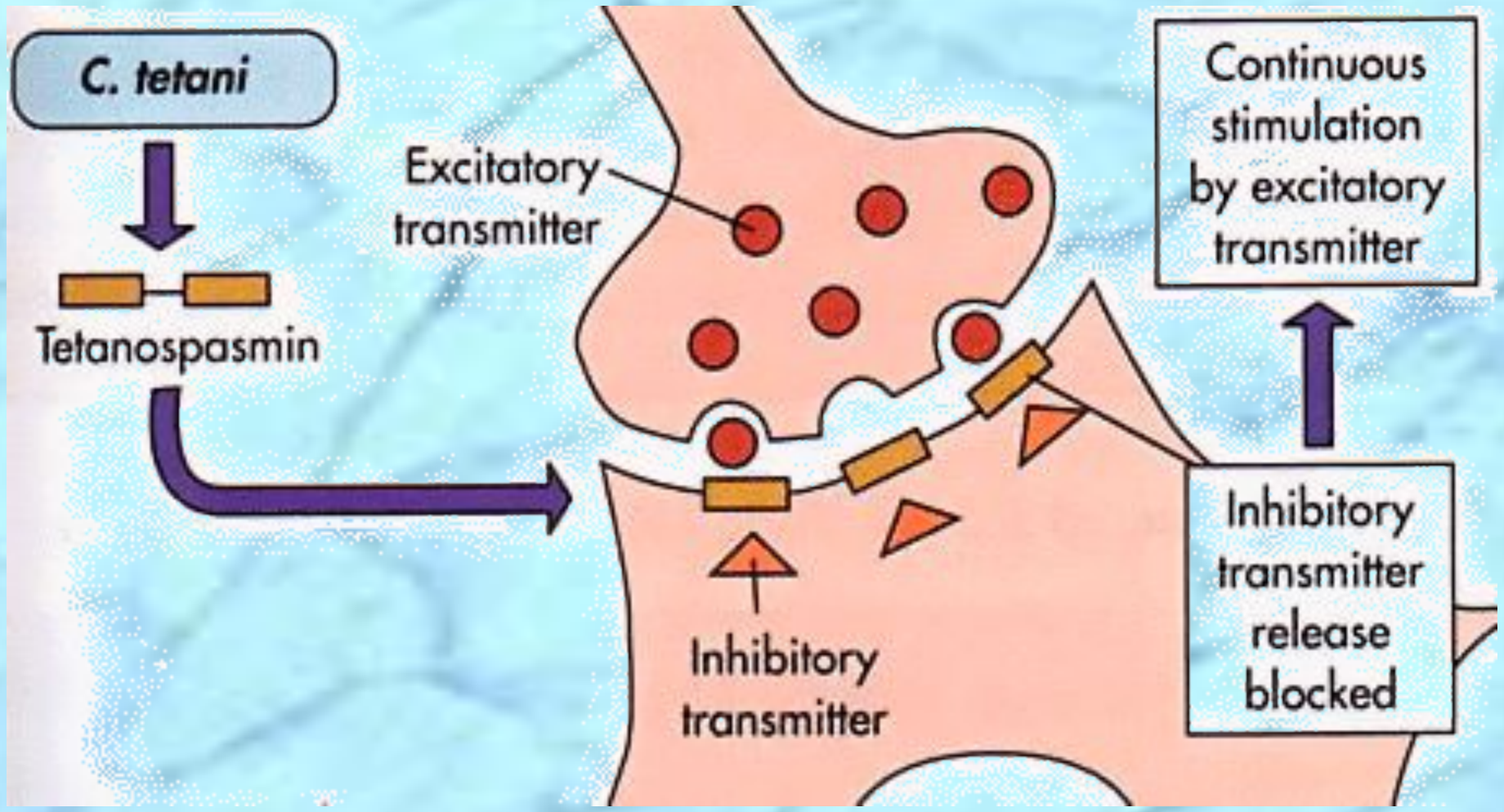
Opisthotonos in Tetanus Patient



Risus Sardonicus in Tetanus Patient



Mechanism of Action of Tetanus Toxin



Clostridium botulinum

Summary of *C. botulinum* Infections

Physiology and Structure

Gram-positive, spore-forming bacillus.

Strict anaerobe (vegetative cells extremely oxygen-sensitive).

Fastidious growth requirements.

Can produce one of seven distinct botulinum toxins (A–G).

Strains associated with human disease produce lipase, digest milk proteins, hydrolyze gelatin, and ferment glucose.

Virulence

Spore formation.

Botulinum toxin (prevents release of neurotransmitter acetylcholine).

Binary toxin.

Epidemiology

Ubiquitous; *C. botulinum* spores are found in soil worldwide

Human diseases associated with toxins A, B, E, and F.

Relatively few cases of botulism in the United States.

Infant botulism more common than other forms.

Summary of *C. botulinum* Infections (cont.)

Diseases

Foodborne botulism.

Infant botulism.

Wound botulism.

Diagnosis

Botulism confirmed by isolating the organism or detecting the toxin in food products or the patient's feces or serum.

Treatment, Prevention, and Control

Treatment involves administration of metronidazole or penicillin, trivalent botulinum antitoxin, and ventilatory support.

Spore germination in foods prevented by maintaining food in an acid pH, by high sugar content (e.g., fruit preserves), or by storing the foods at 4°C or colder.

Toxin is heat-labile so can be destroyed by heating of food for 20 minutes at 80°C.

Infant botulism is associated with consumption of contaminated foods (particularly honey). Infants younger than 1 year should not be given honey or foods containing it.

C. botulinum — **agent of botulism,** a rare, but severe (lethal) neuroparalytic disease

Morphology and Physiology

- heterogeneous group of fastidious, strictly anaerobic bacilli
- **motile by peritrichous flagella**
- heat-resistant **spores (ovoid, subterminal)**
- proteolytic and non-proteolytic

Antigenic Structure

- species divided into **four groups (I-IV)** based on type of toxin produced and proteolytic activity
- **seven antigenically distinct botulinum toxins (types A to G)**
- somatic antigens - heat stable and heat labile; spore antigens - more specific

Pathogenicity Determinants

- **lethal foodbome intoxication with toxin types A,B,E,or F**; shorter incubation period, poor prognosis
- phage-mediated, systemic-acting A-B neurotoxin (botulinum toxin = botulin) released at cell lysis
  - **Mode of Action** - one of most extremely potent neurotoxins known
    (1 ng of purified toxin contains about 200,000 minimal lethal doses (MLDs) for a 20g mouse)
    - **A-B toxin ingested, binds specific receptors on peripheral cholinergic nerve endings (neuromuscular junctions) where it blocks release of presynaptic acetylcholine (excitatory neurotransmitter) blocking muscle stimulation & resulting in flaccid paralysis**
    - **Early**: nausea, vomiting, weakness, lassitude (lack of energy), dizziness, constipation
    - **Later**: double vision, difficulty in swallowing and speaking
    - Final: death due to respiratory paralysis

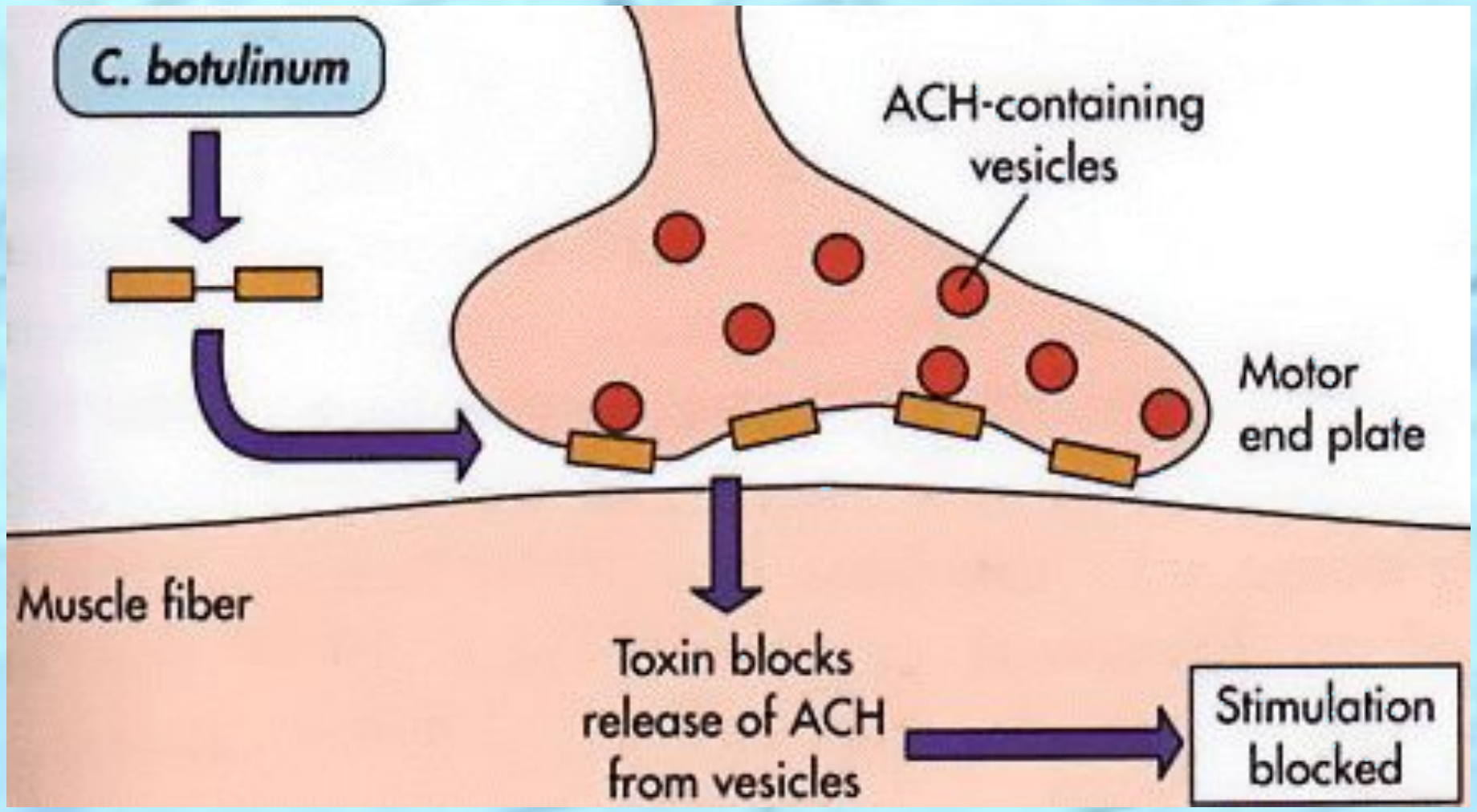
Lab Identification

- microscopic detection or Cx (culture) are often unsuccessful (few organisms and slow growing)
- toxin detected and typed in lab via toxicity and antitoxin neutralization tests in mice or by ELISA

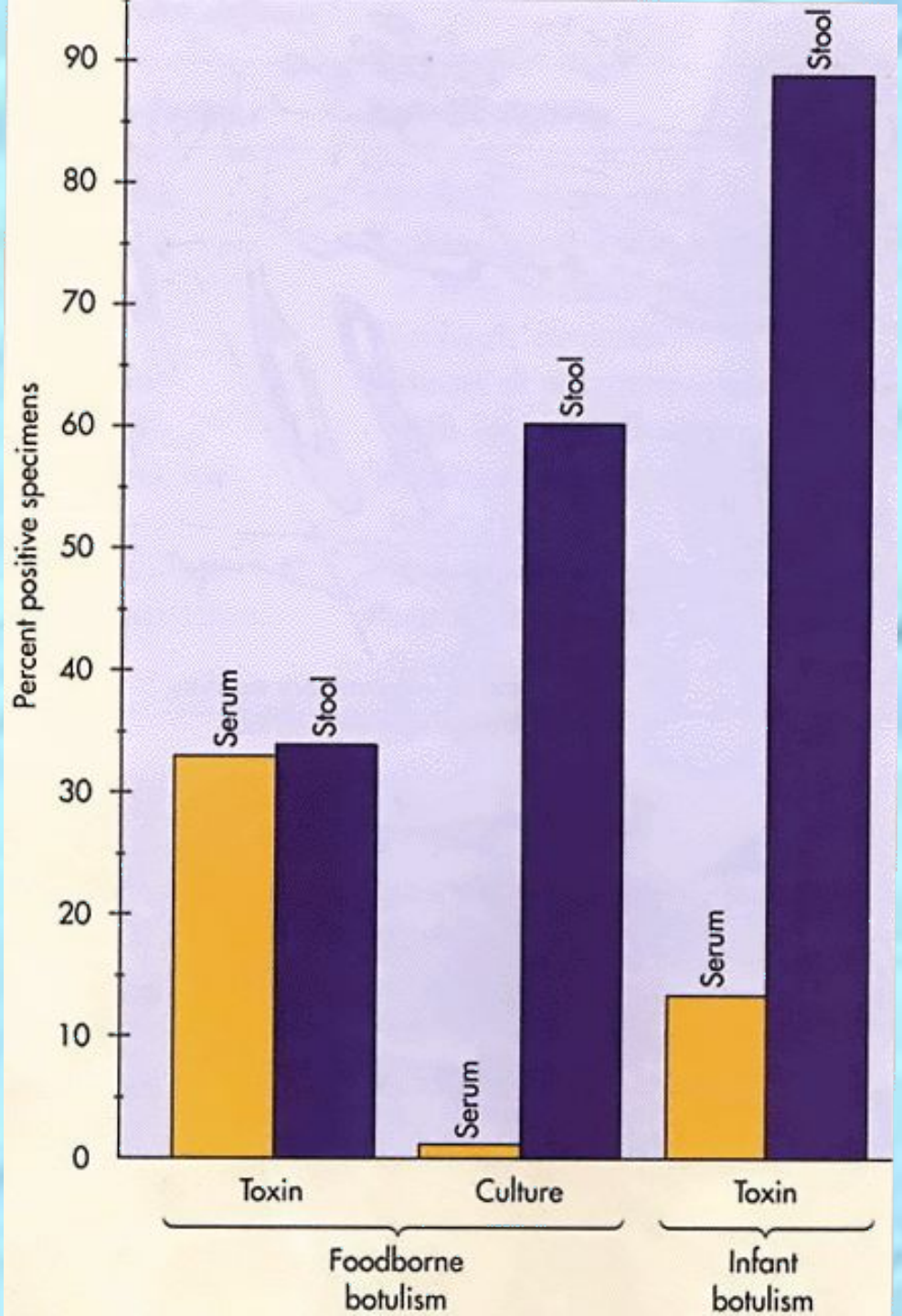
Diagnosis/Treatment/Prevention

- **crucial to rapidly diagnose** (symptoms often confusing); note the type of botulinum toxin involved
- Tx (treatment) should be administered as quickly as possible on basis of clinical Dx (diagnosis)
  - **ventilatory support & trivalent (A, B, E) antitoxin (polyvalent) binds free toxin in bloodstream**
  - administer **gastric lavage & metronidazole or penicillin eliminates organisms from GI tract**
  - **care in home canning** and in heating of home-canned food; toxoid is available

Mechanism of Action of Botulinum Toxin



Rates of Isolation of C. botulinum and Botulinum Toxin



Clostridium dificile

Physiology and Structure

Gram-positive, spore-forming bacillus.

Strict anaerobe (vegetative cells are extremely oxygen-sensitive).

Virulence

Refer to Table 37–5.

Epidemiology

The organism is ubiquitous.

Colonizes the intestines of a small proportion of healthy individuals (<5%).

Exposure to antibiotics is associated with overgrowth of *C. difficile* and subsequent disease (endogenous infection).

Spores can be detected in hospital rooms of infected patients (particularly around beds and in the bathrooms); these can be an exogenous source of infection.

Summary of C. dificile Infections

Summary of C. dificile Infections (cont.)

Diseases

Asymptomatic colonization.

Antibiotic-associated diarrhea.

Pseudomembranous colitis.

Diagnosis

C. difficile disease is confirmed by isolating the organism or detecting the cytotoxin or enterotoxin in the patient's feces.

Treatment, Prevention, and Control

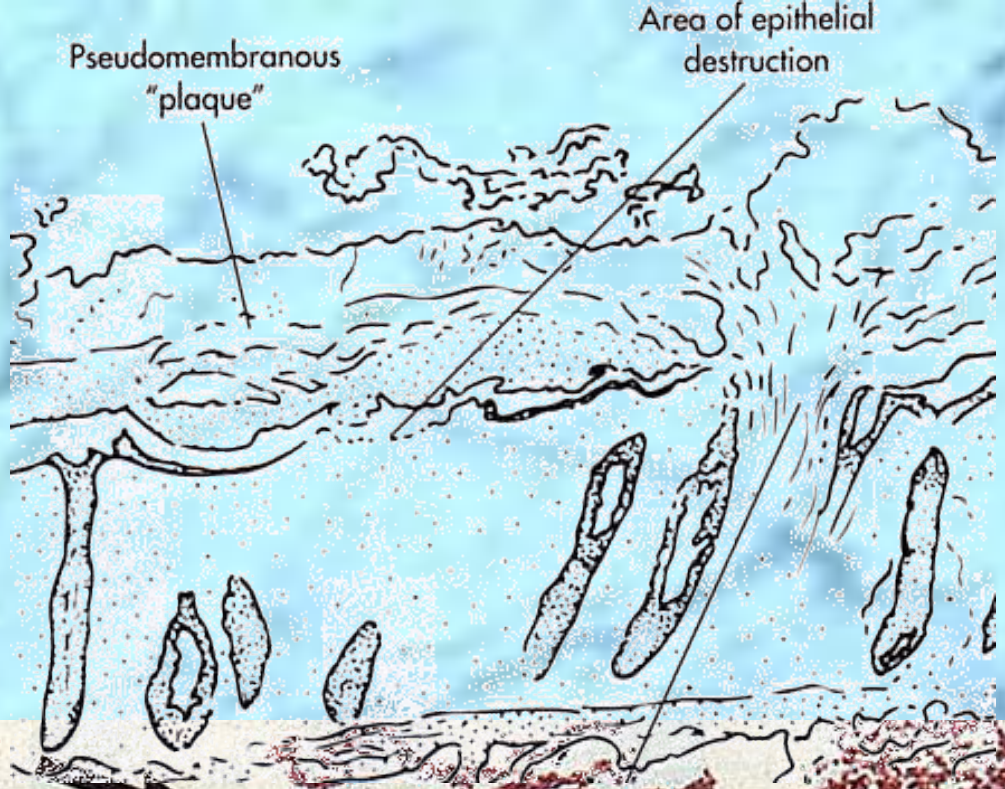
The implicated antibiotic should be discontinued.

Treatment with metronidazole or vancomycin should be used in severe disease.

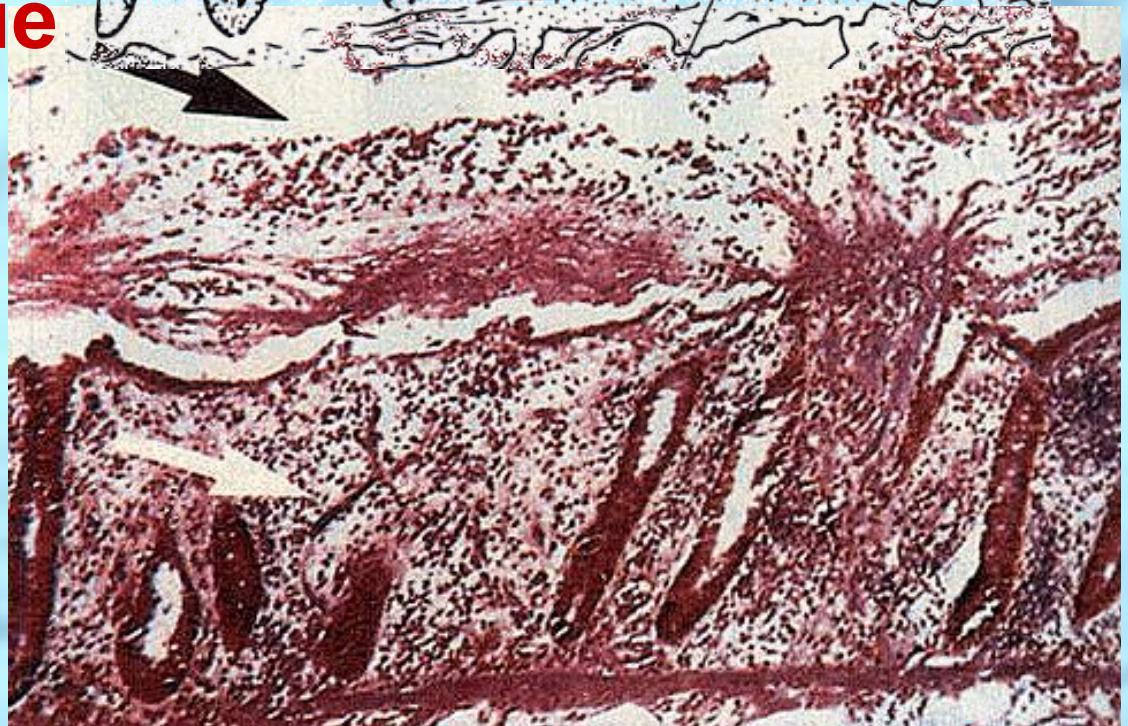
Relapse is common, because the spores are not affected by antibiotics; a second course of therapy with the same antibiotic is usually successful.

The hospital room should be carefully cleaned after the infected patient is discharged.

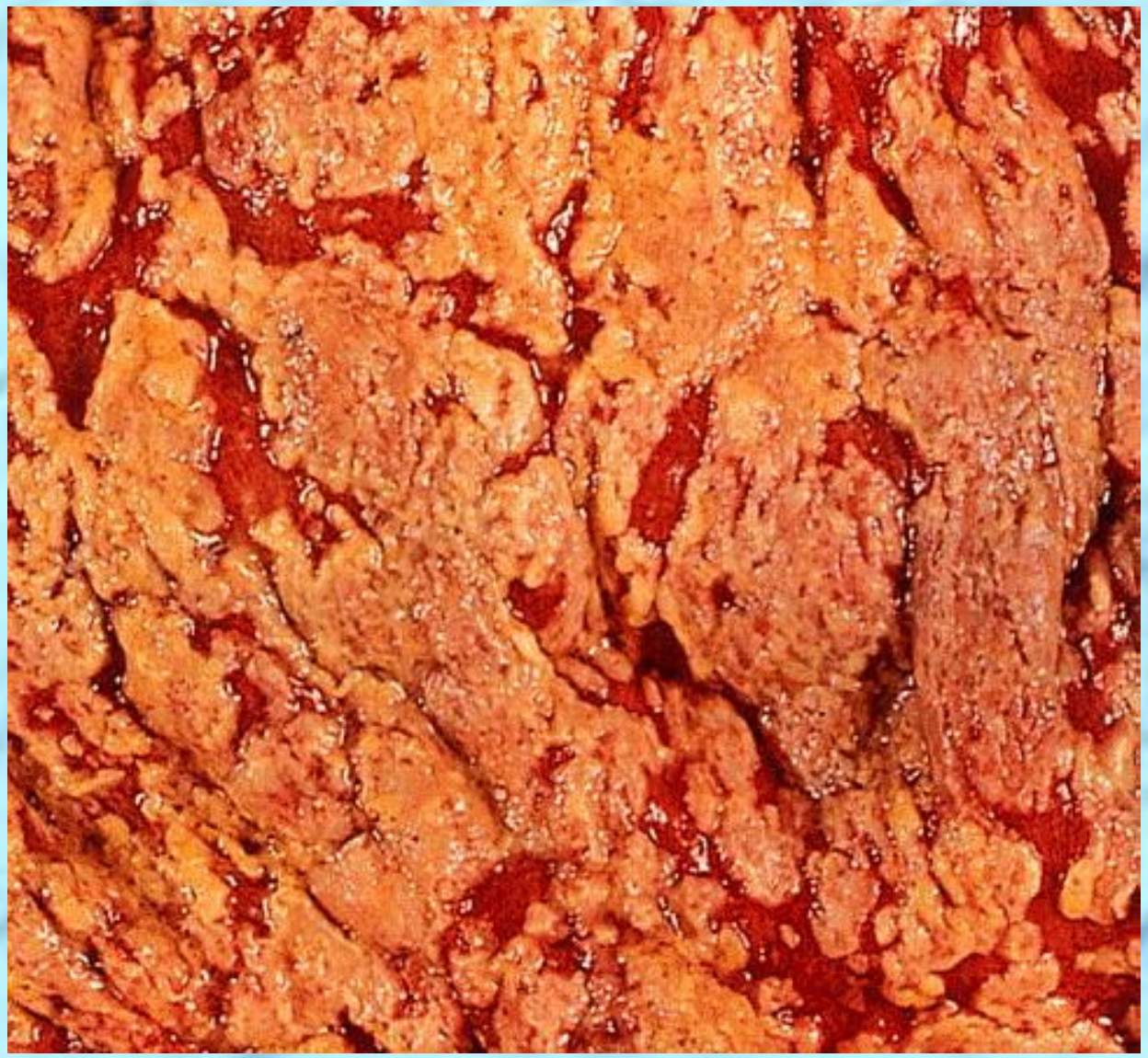
Antibiotic-Associated Colitis



Plaque



Antibiotic-Associated Colitis



C. dificile Virulence Factors

Virulence Factor	Biologic Activity
Enterotoxin (toxin A)	Produces chemotaxis; induces cytokine production with hypersecretion of fluid; produces hemorrhagic necrosis
Cytotoxin (toxin B)	Induces depolymerization of actin with loss of cellular cytoskeleton
Adhesin factor	Mediates binding to human colonic cells
Hyaluronidase	Produces hydrolytic activity
Spore formation	Permits organism's survival for months in hospital environment

Other Clostridium

Virulence Factors Associated with Other Clostridium

Species and Their Virulence Factors	Biologic Activity
C. septicum	
α toxin	Necrotizing, hemolytic toxin
β toxin	Heat-stable deoxyribonuclease
γ toxin	Hyaluronidase
δ toxin	Oxygen-labile hemolysin
Neuraminidase	Alters cell membrane glycoproteins
C. sordellii	
Lecithinase	Phospholipase C
Hemolysin	Oxygen-labile hemolytic activity
Fibrinolysin	Tissue destruction
Lethal β toxin	Necrotic enterotoxin activity
Hemorrhagic toxin	Hemorrhagic cytotoxin activity
C. histolyticum	
α toxin	Necrotizing (not hemolytic) toxin
β toxin	Collagenase
γ toxin	Protease
δ toxin	Elastase
ϵ toxin	Oxygen-labile hemolysin
C. novyi	
α toxin	Necrotizing toxin
β toxin	Lecithinase; necrotizing, hemolytic toxin
γ toxin	Lecithinase; necrotizing, hemolytic toxin
δ toxin	Oxygen-labile hemolysin
ϵ toxin	Lipase
ζ toxin	Hemolysin
η toxin	Tropomyosinase
θ toxin	Lecithinase
C. barati	
Botulinum toxin	Neurotoxin
C. butyricum	
Botulinum toxin	Neurotoxin

REVIEW

Clostridium

Clostridium spp. Anaerobic Gram-Positive Spore-Forming Bacilli

Four broad types of pathogenesis:

1. **Histotoxic group — tissue infections**
   (***C. perfringens* type A**, exogenously acquired more commonly than endogenously)
   (**C. *septicum***; endogenously-acquired)
   a. cellulilitis
   b. myonecrosis
   c. gas gangrene
   d. fasciitis
2. **Enterotoxigenic group — gastrointestinal disease**
   a. clostridial foodbome disease (8-24h after ingestion of large numbers of organisms on con-taminated meat products, spores germinate, enterotoxin produced (***C. perfringens* type A**)
   b. necrotizing enteritis (beta toxin-producing ***C.perfringens* type C**)

   (***C. difficile*** endogenously-acquired or exogenously-acquired person-to-person in hospital)
   c. antibiotic-associated diarrhea
   d. antibiotic-associated pseudomembrane colitis

3. **Tetanus** (exogenously acquired) — C. *tetani* neurotoxin
   a. generalized (most common)
   b. cephalic(primary infection in head, comnnonly ear)
   c. localized
   e. neonatal (contaminated umbilical stump)

4. **Botulism** (exogenously acquired) — *C. botulinum* neurotoxin
   a. foodborne (intoxication, 1-2days incubation period)
   b. infant (ingestion of spores in honey)
   c. wound (symptoms similar to foodborne, but 4 or more days incubation)

REVIEW

See Lecture Handouts

Spores

- *Clostridium* form endospores under **adverse environmental conditions**
- Spores are a **survival** mechanism
- Spores are characterized on the basis of position, size and shape
- Most *Clostridium* spp., including ***C. perfringens*** **and *C. botulinum*, have ovoid subterminal (OST) spores**
- ***C. tetani*** **have round terminal (RT) spores**

REVIEW

Review of Clostridium perfringens

REVIEW

Clostridium perfringens — histotoxic or enterotoxigenic infections

Morphology and Physiology

- **large, rectangular bacilli** (rod) staining **gram-positive**
- **spores rarely seen** *in vitro* or in clinical specimens (ovoid, subterminal)
- **non-motile,** but **rapid spreading growth** on blood agar mimics growth of motile organisms
- aerotolerant, especially on media supplemented with blood
- grow at temperature of 20-50°C (optimum 45°C) and pH of 5.5-8.0

Pathogenicity Determinants (note that toxins include both cytolytic enzymes and bipartite exotoxins)

- four **major lethal toxins** (alpha (α), beta (β), epsilon (ε), and iota (ι) toxins) and an **enterotoxin**
- **six minor toxins** (delta(δ), theta(θ), kappa(κ), lambda(λ), mu(μ), nu(η)toxins) **& neuraminadase**
- C. *perfringens* subdivided into five types (A-E) on basis of production of major lethal toxins
- **C. *perfringens* Type A** (only major lethal toxin is alpha toxin) responsible for histotoxic and enterotoxigenic infections in humans; Type C causes necrotizing enteritis (not in U.S.)

Lab Identification

- direct smear and Gram stain, capsules upon direct examination of wound smears
- culture takes advantage of **rapid growth** in chopped meat media at 45° C to enrich and then isolate onto blood agar streak plate after four to six hours
- **gas** from glucose fermentation
- in vivo toxicity testing and identification of the specific toxin types involved
- **double zone of hemolysis** on blood agar (p-hemolytic theta(e) toxin, a-hemolytic alpha(oc) toxin)
- **Nagler rxn;** precipitation in serum or egg yolk media; oc -toxin (phospholipase C) is a lecithinase
- **"stormy" fermentation** (coagulaltion) of milk due to large amounts of acid and gas from lactose

Diagnosis/Treatment of systemic infection — Early diagnosis and aggressive treatment essential

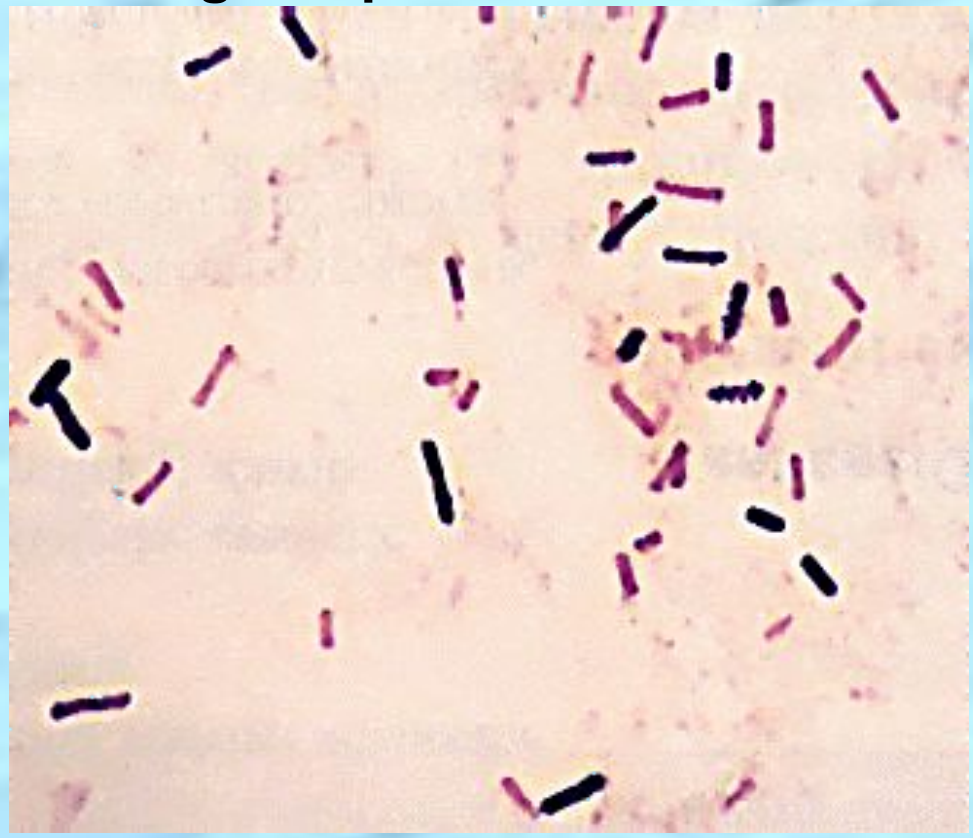
- removal of necrotic tissue **(surgical debridement)**
- **Penicillin G in high doses** if more serious infection

  Of poorly defined clinical value are:
  - administration of antitoxin
  - hyperbaric oxygen (dive chamber) adjunct therapy (??inhibit growth of anaerobe??)

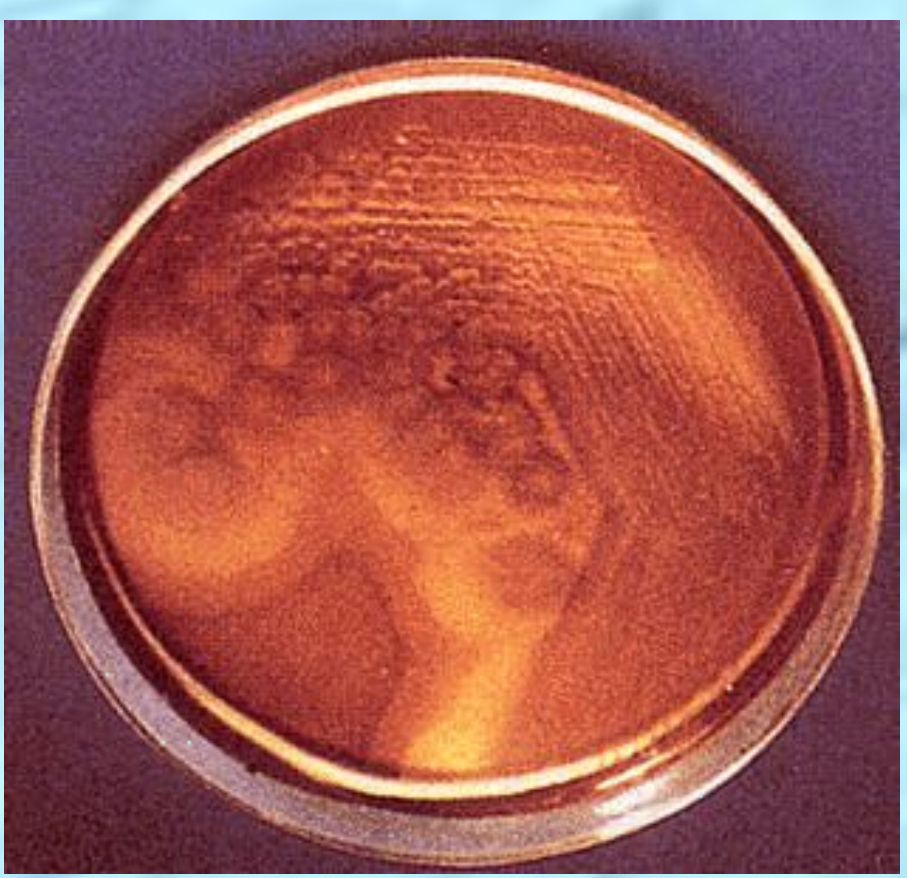
REVIEW

Micro & Macroscopic C. perfringens

NOTE: Large rectangular gram-positive bacilli



NOTE: Double zone of hemolysis



Inner beta-hemolysis = θ toxin
Outer alpha-hemolysis = α toxin

REVIEW

C. perfringens Virulence Factors

Virulence Factors	Biologic Activity
Major	
α toxin	Lethal toxin; phospholipase C (lecithinase); increases vascular permeability; hemolysin; produces necrotizing activity
β toxin	Lethal toxin; necrotizing activity
ϵ toxin	Lethal toxin; permease
ι toxin	Lethal binary toxin; necrotizing activity; adenosine diphosphate (ADP) ribosylating
Minor	
δ toxin	Hemolysin
θ toxin	Heat- and oxygen-labile hemolysin; cytolytic
κ toxin	Collagenase; gelatinase; necrotizing activity
λ toxin	Protease
μ toxin	Hyaluronidase
ν toxin	Deoxyribonuclease; hemolysin; necrotizing activity
Enterotoxin	Alters membrane permeability (cytotoxic, enterotoxic)
Neuraminidase	Alters cell surface ganglioside receptors; promotes capillary thrombosis

REVIEW

Exotoxins Associated with C. perfringens Types A-E

Type of Isolate	Major Lethal Toxins			
	α	β	ϵ	ι
A	+	−	−	−
B	+	+	+	−
C	+	+	−	−
D	+	−	+	−
E	+	−	−	+

REVIEW

C. perfringens Nagler Reaction

NOTE: **Lecithinase (α-toxin; phospholipase) hydrolyzes phospholipids in egg-yolk agar around streak on right. Antibody against α-toxin inhibits activity around left streak.**

REVIEW

Review of Clostridium tetani

Clostridium tetani — agent of tetanus

Morphology and Physiology-

- long thin gram-positive organism that stains gram negative in old cultures
- **round terminal spore** gives drumstick appearance
- motile by peritrichous flagella
- grow on blood agar or cooked meat medium with swarming
- beta-hemolysis exhibited by isolated colonies
- spores resist boiling for 20 minutes

Antigenic Structure-

flagella (H), somatic (0), and spore antigens. Single antigenic toxin characterizes all strains.

Pathogenicity Determinants"

- play a role in local infection only in conjunction with other bacteria that create suitable environment for their invasion
- systemic-acting, **plasmid-mediated A-B neurotoxin (tetanospasmin)** produced intracellularly
  - Mode of Action — one of most poisonous substances
    - binds gangliosides in synaptic membranes (synapses of neuronal cells) and **blocks release of inhibitory neurotransmitters;** continuous stimulation by excitatory transmitters
    - muscle spasms (spastic paralysis) (trismus (lockjaw), risus sardonicus, opisthotonos), cardiac arrhythmias, fluctuations in blood pressure

Lab Identification"

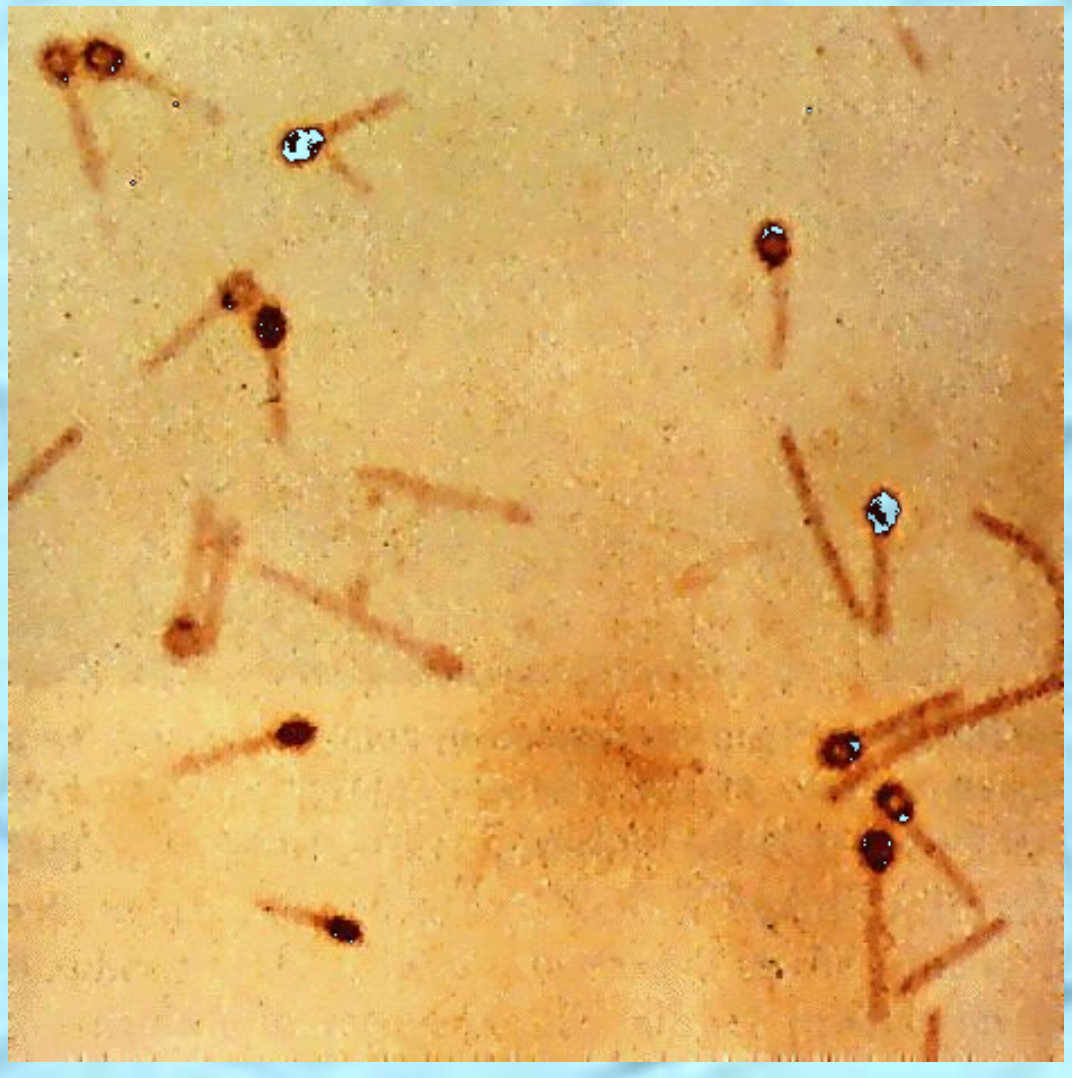
- use characteristics of resistance to heat, motility, and toxin production to help identify

Diagnosis/Treatment/Prevention

- empirical diagnosis on basis of clinical manifestations
- treat to prevent elaboration and absorption of toxin
  - **clean wound** (debridement), **control spasms**
  - **metronidazole** administered to eliminate vegetative bacteria that produce neurotoxin
  - **passive immunity** (human tetanus immunoglobulin); **vaccination** (active) as preventative
  - **antitoxin** administered to bind free tetanospasmin

REVIEW

Clostridium tetani Gram Stain



NOTE: **Round terminal spores give cells a "drumstick" or "tennis racket" appearance.**

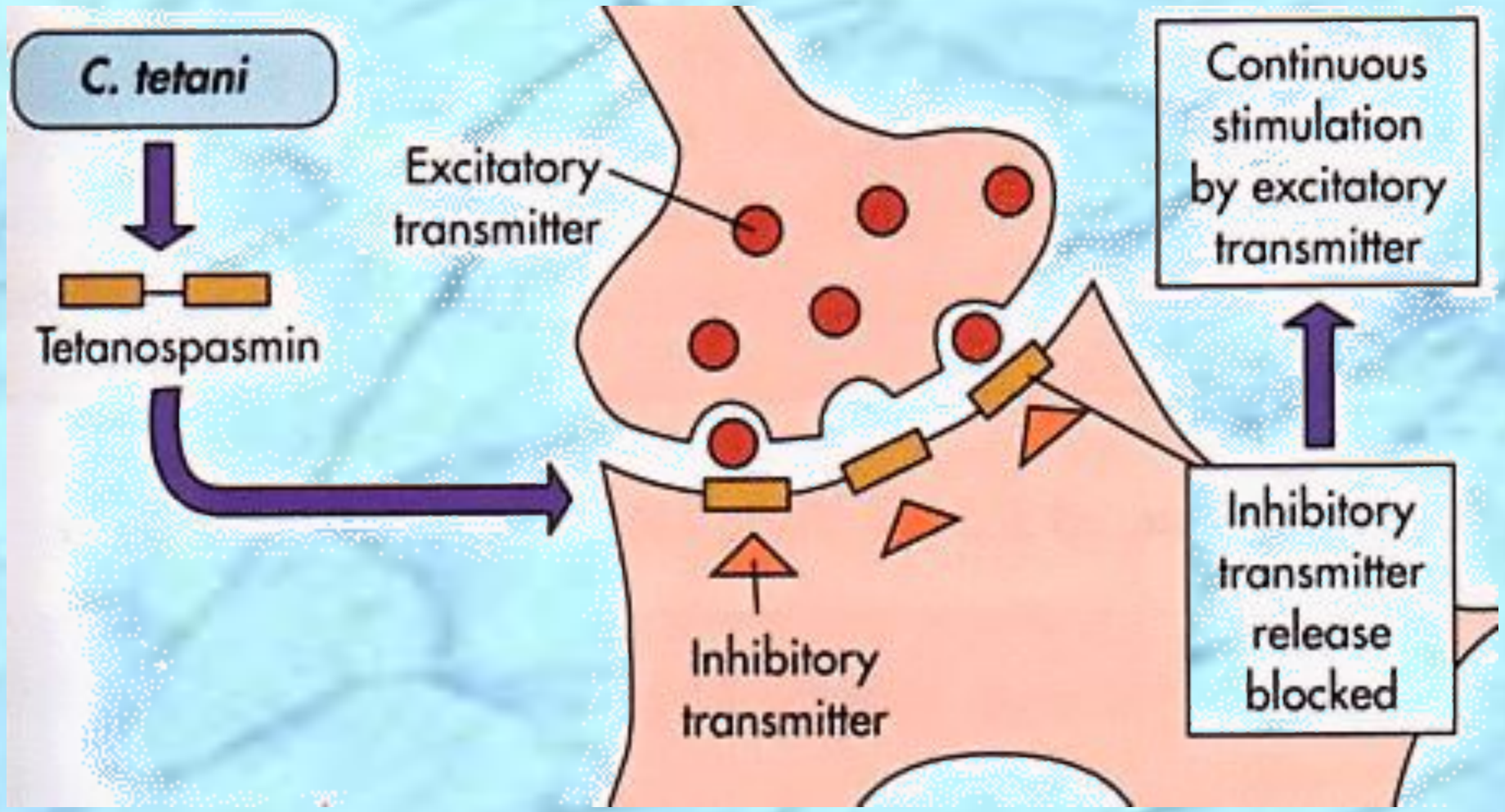
REVIEW

Clinical Forms of Tetanus

Disease	Clinical Manifestations
Generalized	Involvement of bulbar and paraspinal muscles (trismus or lockjaw, risus sardonicus, difficulty swallowing, irritability, opisthotonos); involvement of autonomic nervous system (sweating, hyperthermia, cardiac arrhythmias, fluctuations in blood pressure)
Cephalic	Primary infection in head, particularly ear; isolated or combined involvement of cranial nerves, particularly seventh cranial nerve; very poor prognosis
Localized	Involvement of muscles in area of primary injury; infection may precede generalized disease; favorable prognosis
Neonatal	Generalized disease in neonates; infection typically originates from umbilical stump; very poor prognosis in infants whose mothers are nonimmune

REVIEW

Mechanism of Action of Tetanus Toxin



REVIEW

Review of Clostridium botulinum

C. botulinum — **agent of botulism,** a rare, but severe (lethal) neuroparalytic disease

Morphology and Physiology

- heterogeneous group of fastidious, strictly anaerobic bacilli
- **motile by peritrichous flagella**
- heat-resistant **spores (ovoid, subterminal)**
- proteolytic and non-proteolytic

Antigenic Structure

- species divided into **four groups (I-IV)** based on type of toxin produced and proteolytic activity
- **seven antigenically distinct botulinum toxins (types A to G)**
- somatic antigens - heat stable and heat labile; spore antigens - more specific

Pathogenicity Determinants

- **lethal foodbome intoxication with toxin types A, B, E, or F**; shorter incubation period, poor prognosis
- phage-mediated, systemic-acting A-B neurotoxin (botulinum toxin = botulin) released at cell lysis
  - **Mode of Action** - one of most extremely potent neurotoxins known
    (1 ng of purified toxin contains about 200,000 minimal lethal doses (MLDs) for a 20g mouse)
    - **A-B toxin ingested, binds specific receptors on peripheral cholinergic nerve endings (neuromuscular junctions) where it blocks release of presynaptic acetylcholine (excitatory neurotransmitter) blocking muscle stimulation & resulting in flaccid paralysis**
    - **Early**: nausea, vomiting, weakness, lassitude (lack of energy), dizziness, constipation
    - **Later**: double vision, difficulty in swallowing and speaking
    - Final: death due to respiratory paralysis

Lab Identification

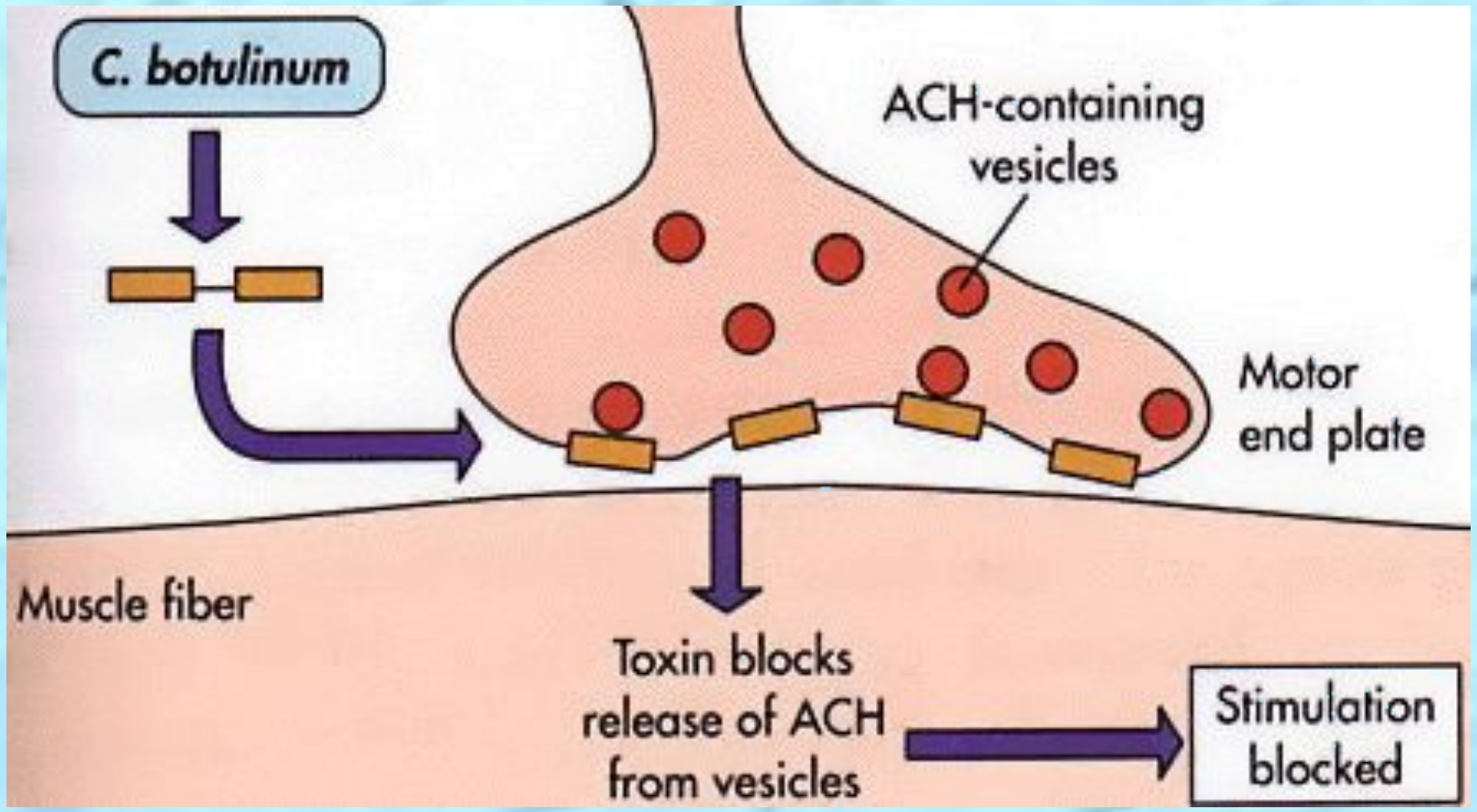
- microscopic detection or Cx (culture) are often unsuccessful (few organisms and slow growing)
- toxin detected and typed in lab via toxicity and antitoxin neutralization tests in mice or by ELISA

Diagnosis/Treatment/Prevention

- **crucial to rapidly diagnose** (symptoms often confusing); note the type of botulinum toxin involved
- Tx (treatment) should be administered as quickly as possible on basis of clinical Dx (diagnosis)
  - **ventilatory support & trivalent (A, B, E) antitoxin (polyvalent) binds free toxin in bloodstream**
  - administer **gastric lavage & metronidazole or penicillin eliminates organisms from GI tract**
  - **care in home canning** and in heating of home-canned food; toxoid is available

REVIEW

Mechanism of Action of Botulinum Toxin



REVIEW

Review of Clostridium dificile

Physiology and Structure

Gram-positive, spore-forming bacillus.

Strict anaerobe (vegetative cells are extremely oxygen-sensitive).

Virulence

Refer to Table 37–5.

Epidemiology

The organism is ubiquitous.

Colonizes the intestines of a small proportion of healthy individuals (<5%).

Exposure to antibiotics is associated with overgrowth of *C. difficile* and subsequent disease (endogenous infection).

Spores can be detected in hospital rooms of infected patients (particularly around beds and in the bathrooms); these can be an exogenous source of infection.

Summary of C. dificile Infections

REVIEW

Summary of C. dificile Infections (cont.)

Diseases

Asymptomatic colonization.

Antibiotic-associated diarrhea.

Pseudomembranous colitis.

Diagnosis

C. difficile disease is confirmed by isolating the organism or detecting the cytotoxin or enterotoxin in the patient's feces.

Treatment, Prevention, and Control

The implicated antibiotic should be discontinued.

Treatment with metronidazole or vancomycin should be used in severe disease.

Relapse is common, because the spores are not affected by antibiotics; a second course of therapy with the same antibiotic is usually successful.

The hospital room should be carefully cleaned after the infected patient is discharged.

REVIEW