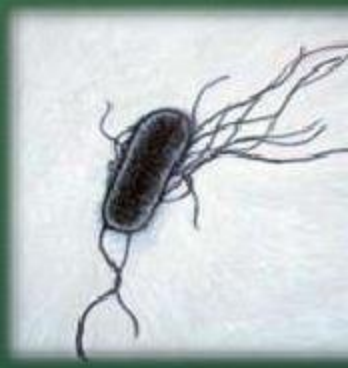
# LECTURE IX

Causative agents of gastro-intestinal diseases (genera *Escherichia*, *Shigella*, *Salmonella*, *Vibrio*, *Campylobacter*,*Helicobacter*). Pathogenic anaerobes ( genus *Clostridium* and *Bacteroides)*.

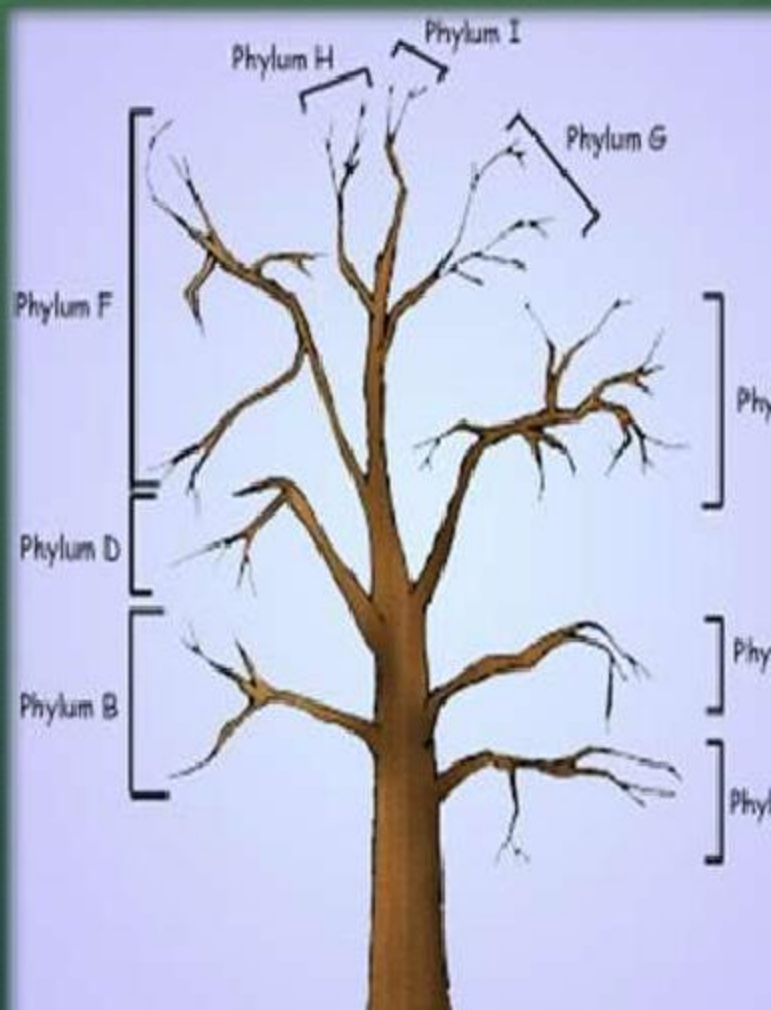
# E.coli

- Morphology Gram - ve Straight rods,
- 1-3 X 0.4 -0.7 microns,
- Appear in singles or in pairs,
- Motile by peritrichate flagella.
- Very few strains non motile
- Not spore forming, Non acid fast.

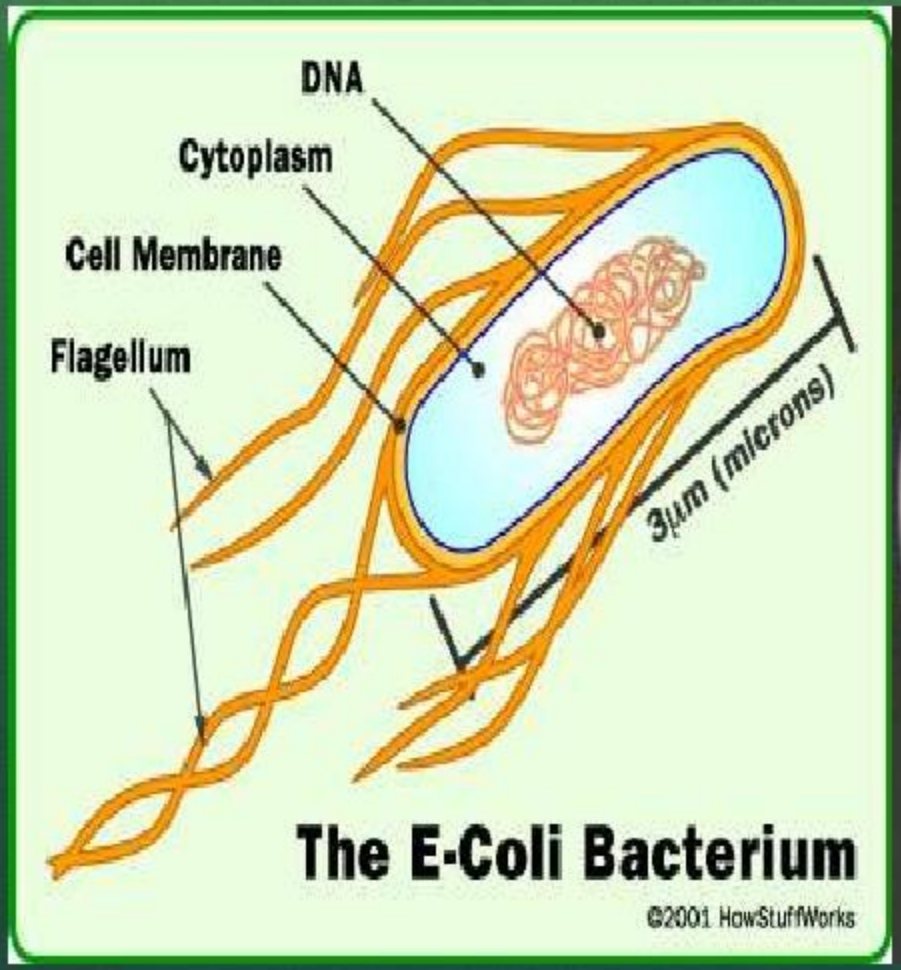


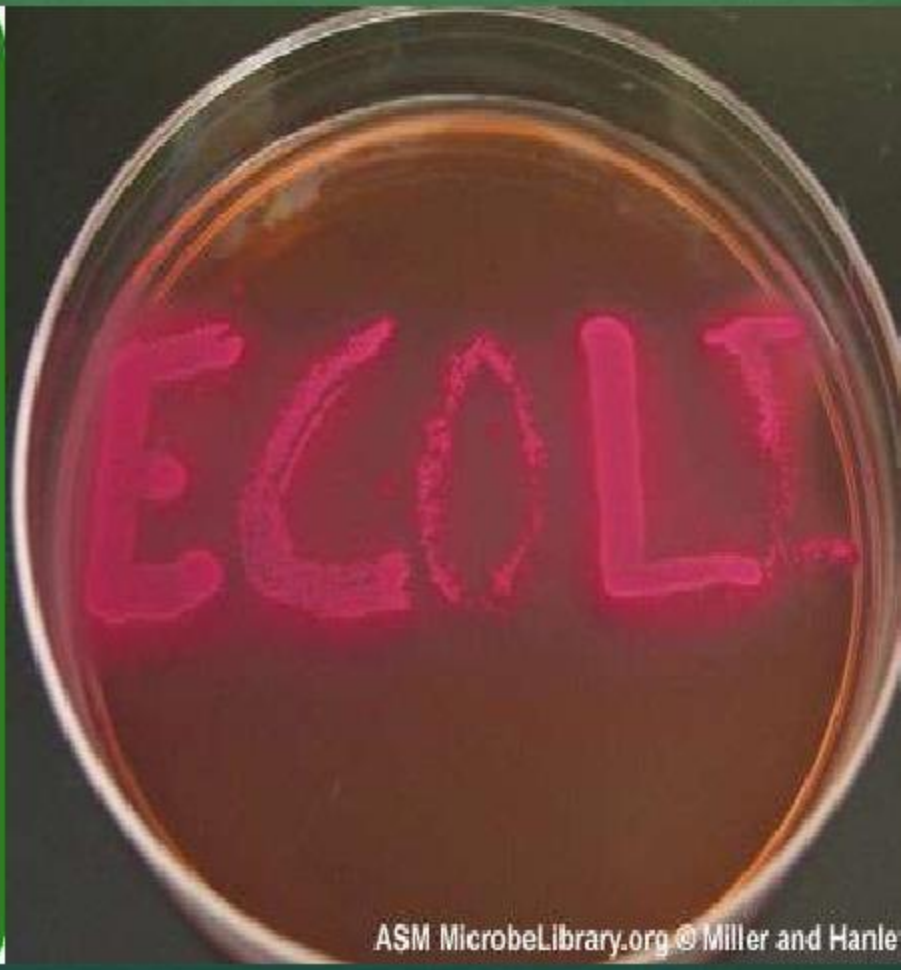
# Classification

- Domain: Bacteria
- Kingdom: Bacteria
- Phylum: Proteobacteria
- Class: Gamma Proteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: Escherichia
- Species: Escherichia coli (E. coli)



# E.coli


DNA
Cytoplasm
Cell Membrane
Flagellum
3µm (microns)
The E-Coli Bacterium
©2001 HowStuffWorks


E.COLI
ASM MicrobeLibrary.org © Miller and Hanle

# Identification of *Enterobacteriaceae* Biochemical reactions

- Oxidase test
  - **All members of *Enterobacteriaceae* are oxidase negative**
  - ***Pseudomonas* is oxidase positive**
- O/F test
  - **All members of *Enterobacteriaceae* are O+/F+**
  - ***Pseudomonas* is O+/F-**
- Nitrate reductase
  - All members of Enterobacteriaceae are nitrate reductase positive
  - *Pseudomonas* is nitrate reductase negative

# E.coli
# Biochemical Characters,

Glucose,Lactose,Mannitol,Maltose

fermented. with A/G

I,M,Vi,C tests.

Indole +

Methyl Red +

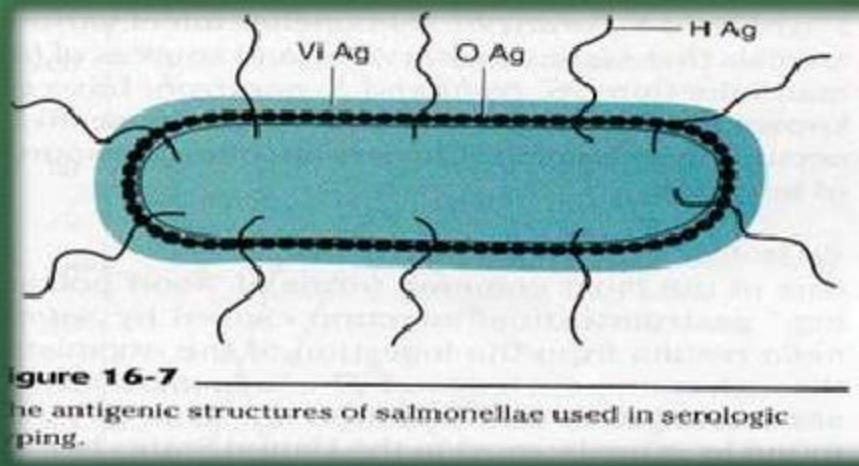
Voges Proskauer – ve I,M,Vi,C tests.

Citrate –ve

Urease not produced.

# E.coli
# Antigenic Structure

- Somatic 0 170
- Capsular K 100
- Flagella H 75
- Virulence factors



igure 16-7
he antigenic structures of salmonellae used in serologic
ping.

Surface Antigens Toxins

O Endotoxic activity

K protects against the phagocytosis

Fimbriae promote virulence ( important in UTI )

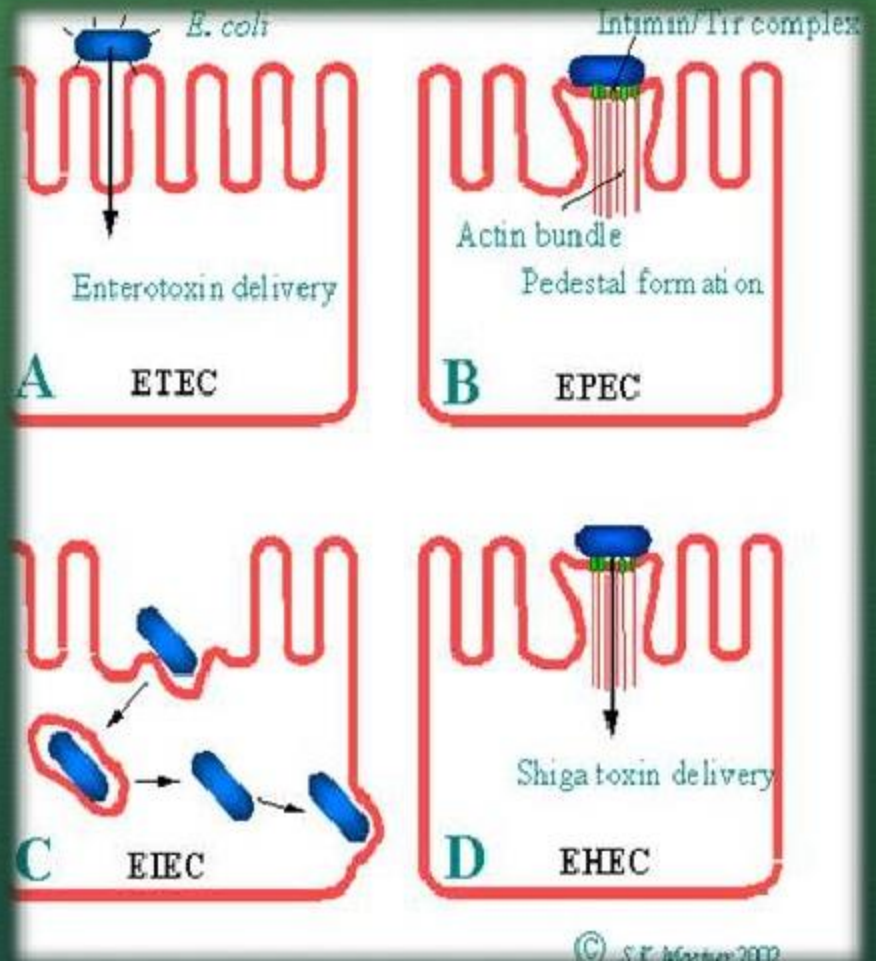
# Toxins and E.coli

- E.coli produce Exotoxins
- Hemolysins, Enterotoxins causes Diarrheas,
- Important toxins produces.
- Heat labile HL Heat stable HS

**Vero toxins VT Like Shigella toxins**

# Mechanism of action of Toxins

- Increased cAMP alters the activity of sodium and chloride transporters producing an ion imbalance that results in fluid transport into the bowel



# Classification of E.coli

1. Enteropathogenic EPEC
2. Enterotoxigenic ETEC
3. Enteroinvasive EIEC
4. Enterohemorrhagic EHEC
5. Enteroaggresive EAEC

# Enteropathogenic E.coli

- Causes diarrheal disease in children,
- EPEC O26/O11
- Produce Verocytotoxin
- Infantile enteritis, Involves upper part of Intestine
- Brush border of the intestine is lost
- Intimacin – EPEC adhesion factor.
- Frequent in summer months
- Poor hygiene predisposes.

# Enterotoxigenic E.coli

- Causes travelers diarrhea
- Water contaminated with Human and Animal feces predisposes.
- Laboratory Diagnosis

Demonstration of Enterotoxin LT and ST

Tissue culture tests,

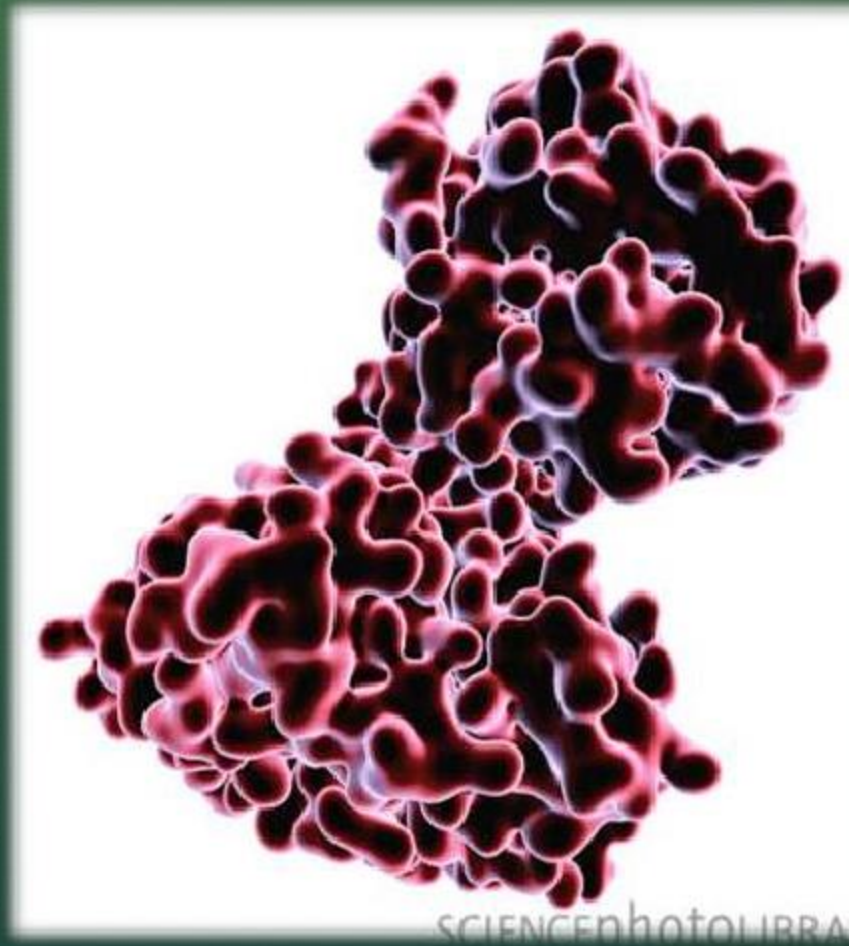
ELISA

Passive agglutination tests.

Animal experiments in Rabbit ileal loop test.

# Entero invasive E.coli

- Some are non motile strains,
- Atypical resembles like Shigella.
- Clinically mild diarrhea
- Sereny test positive animal Rabbit.
- ELISA



# EHEC ( contd )

- Culture
- DNA detection methods.
- Cytotoxic effects on Vero cells.
- Detection with monovalent sera O157/H7

# Enterohemorrhagic bacteria Escherichia coli (EHEC)

Most Escherichia coli (E.coli) strains are harmless.

But some, like enterohemorrhagic **E. coli (EHEC)**, are a hazard to human health and life.

**Incubation period:**

**three to eight days**

E. coli (EHEC), once in th human stomach, begins p ing toxins that cause serio illnesses

**Symptoms caused by E. coli (EHEC)**

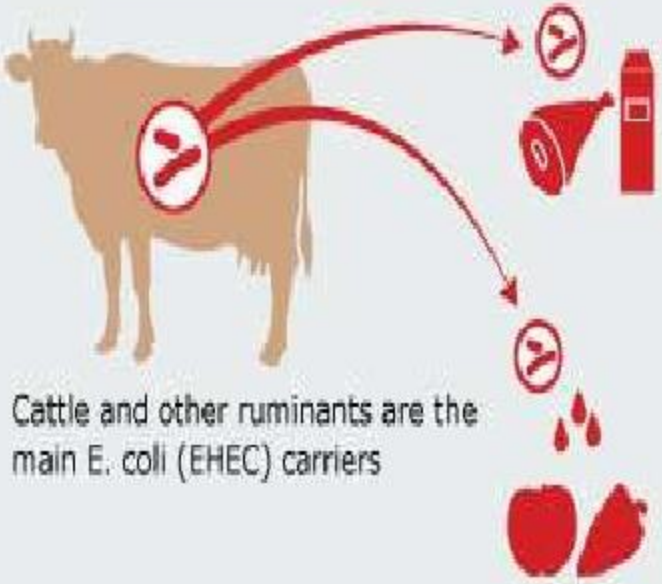
- Stomach muscle spasm
- Diarrhea (sometimes bl diarrhea)
- Fever
- Vomiting

**Complications:**

hemolytic uremic syndrom

**Death rate:** 3-5 %

## INFECTION SOURCES



Cattle and other ruminants are the main E. coli (EHEC) carriers

Uncooked meat and raw milk

*The bacteria die when food is exposed to heat (70°C and higher)*

**Fruit and vegetables** (droppings of sick animals find their way into water bodies that in turn feed the soil)

# Enteroaggresive E.coli EAEC

- Can cause Diarrhea Detect by Culture methods
- Brick-like aggregates on cell surfaces
- Mucus biofilm inhibits fluid absorption
- Diarrhea
- Detection of Enterotoxin

# Culturing for E.coli

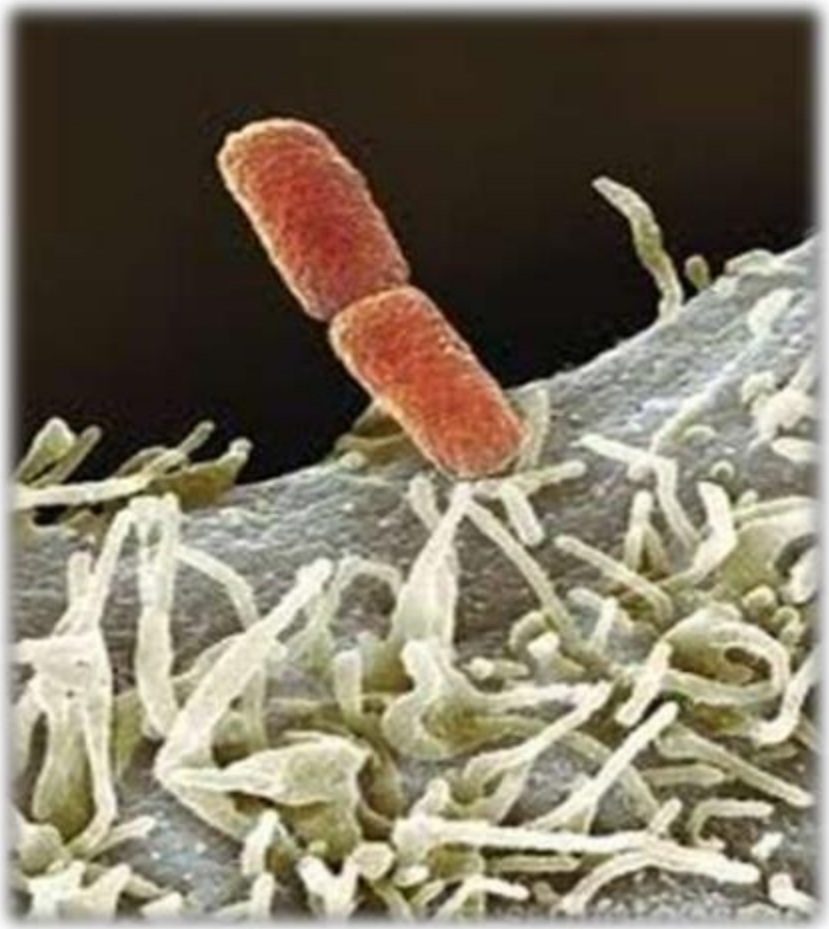
- Mid stream sample/semi quantitative culturing (Kass et al ) >_ 1.00,000/ml of urine. ( significant Bacteriuria )
- Urine should not be kept in wards for > 2 hours and to be preserved at 4 c
- Culture by standard loop method.
- Fixed volume cultured on MacConkey agar Lactose fermenters I M Vi C
- Antibiotic sensitivity tested

# Shigella a Highly Infectious Bacteria

- *Shigella* is one of the most infectious of bacteria and ingestion of as few as **100-200 organisms** will cause disease.
- Most individuals are infected with shigellae when they ingest food or water contaminated with human fecal material.
- Shigella can survive up to 30 days in milk, eggs, cheese or shrimps.

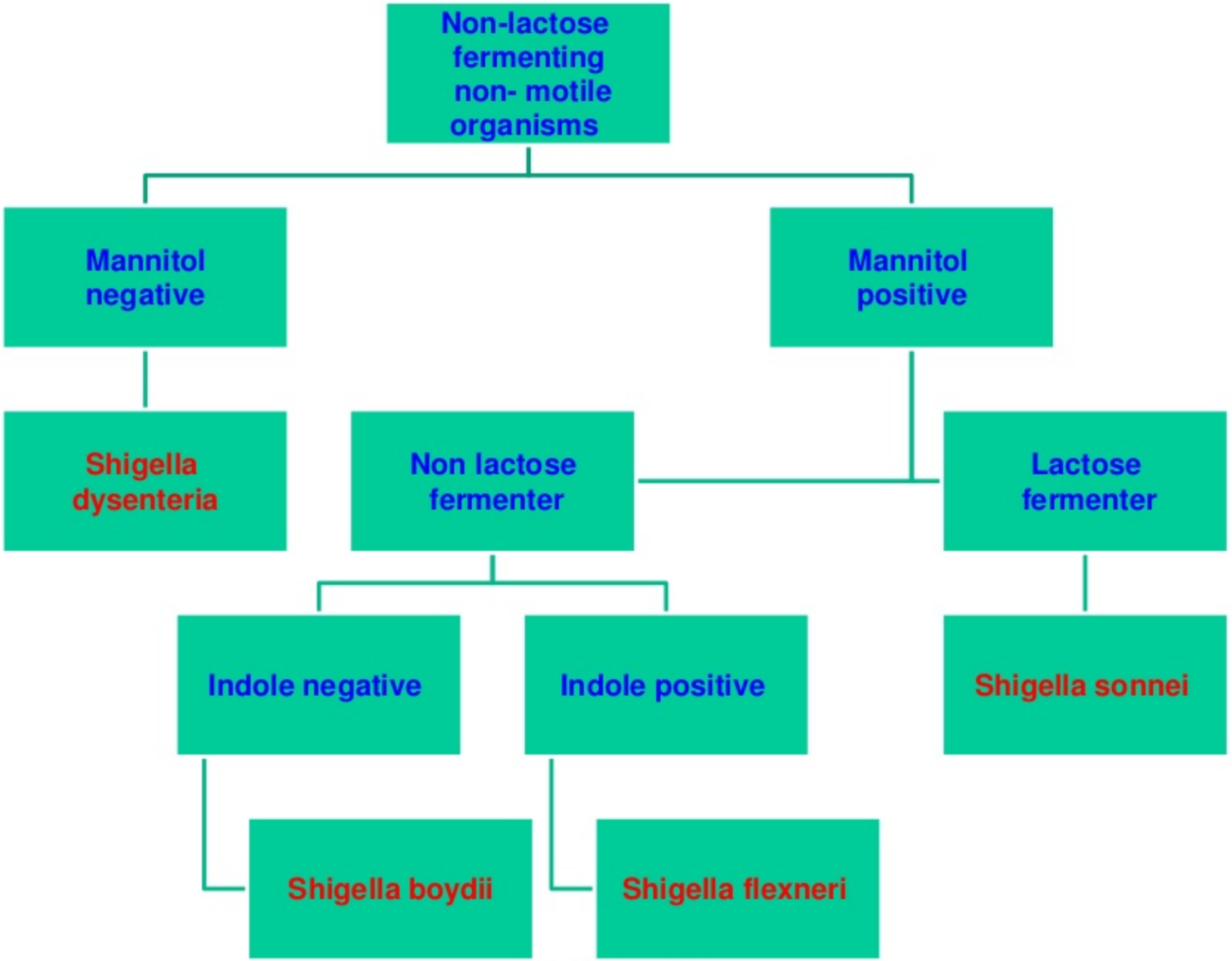
# MORPHOLOGY AND STAINING:

- Short rods
- - Non-encapsulated
- - Non-motile
- - Non-spore former
- - Gram-negative



# CLASSIFICATION
## on Basis of Mannitol Fermentation:

- 1. Non-mannitol-fermenters
- *Shigella dysenteria*
- 2. Mannitol-fermenters
- *Shigella flexneri*
- *Shigella boydii*
- *Shigella sonnei*


Non-lactose fermenting non- motile organisms
Mannitol negative
Mannitol positive
Shigella dysenteria
Non lactose fermenter
Lactose fermenter
Indole negative
Indole positive
Shigella sonnei
Shigella boydii
Shigella flexneri

# Pathogenesis & Immunity

- **Exotoxin (Shiga toxin)** is neurotoxic, cytotoxic, and enterotoxic, encoded by chromosomal genes,
- **Enterotoxic effect**: Shiga toxin adheres to small intestine receptors
- Blocks absorption (uptake) of electrolytes, glucose, and amino acids from the intestinal lumen

# Characteristics of Shiga Toxin

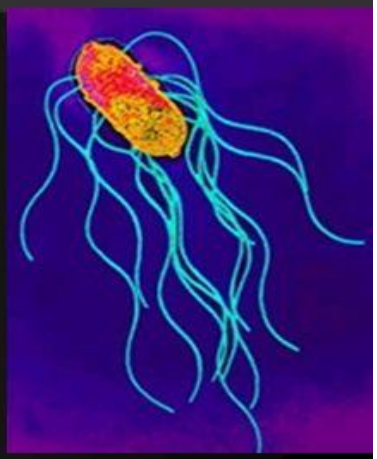
- Enterotoxic, neurotoxic and cytotoxic
- Encoded by chromosomal genes
- Two domain (A-5B) structure
- Similar to the Shiga-like toxin of enterohemorrhagic *E. coli* (EHEC)
  - NOTE: except that Shiga-like toxin is encoded by lysogenic bacteriophage

# Growth on Selective Medium

- In XLD they appear pinkish to reddish colonies while in Heaktoen Enteric Agar (HEA), they give green to blue green colonies.


Shigella on EMB
Shigella on MacConkey
Shigella on ENDO
Shigella on Hektoen
Shigella on SS agar

# SALMONELLA



# Classification

- Based on DNA-DNA hybridization: 2 species

a. *Salmonella enterica* and
b. *Salmonella bongori*

*Salmonella enterica* comprises 6 sub species

*S. enterica* subspecies *enterica*

subspecies *salamae*

subspecies *arizonae*

subspecies *diarizonae*

subspecies *houtanae*

subspecies *indica*

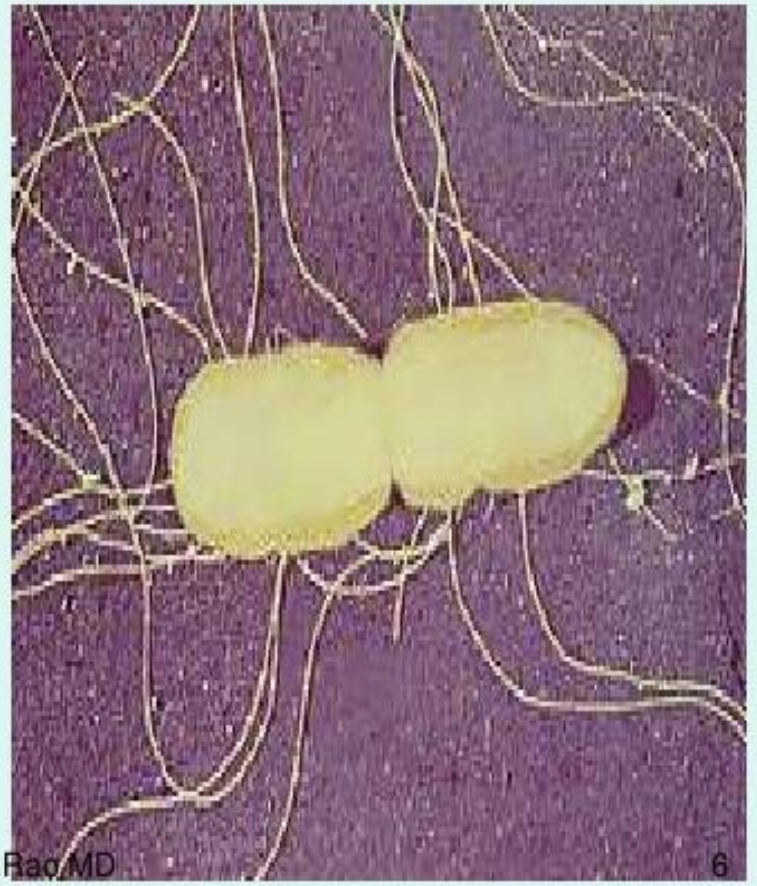
# Morphology

- Gram-negative rods
- Motile
- Nonsporing, noncapsulated meas. 2-4 x0.6 micron

- Family: Enterobacteriaceae
- Gram-negative rods
- Motile except *Salmonella* Gallinarum and *S.* Pullorum
- Aerobic and facultatively anaerobic
- Catalase positive; oxidase negative
- Attack sugars by fermentation and produces gas
- Citrate utilization usually positive except *S.* Typhi and *S.* Paratyphi A
- Lysine decarboxylase usually positive except *S.* Paratyphi A
- G+C content 50-53 mol%

# Morphology of Salmonella

- Gram negative bacilli
- 1-3 / 0.5 microns,
- Motile by peritrichous flagella



# Cultural Characters

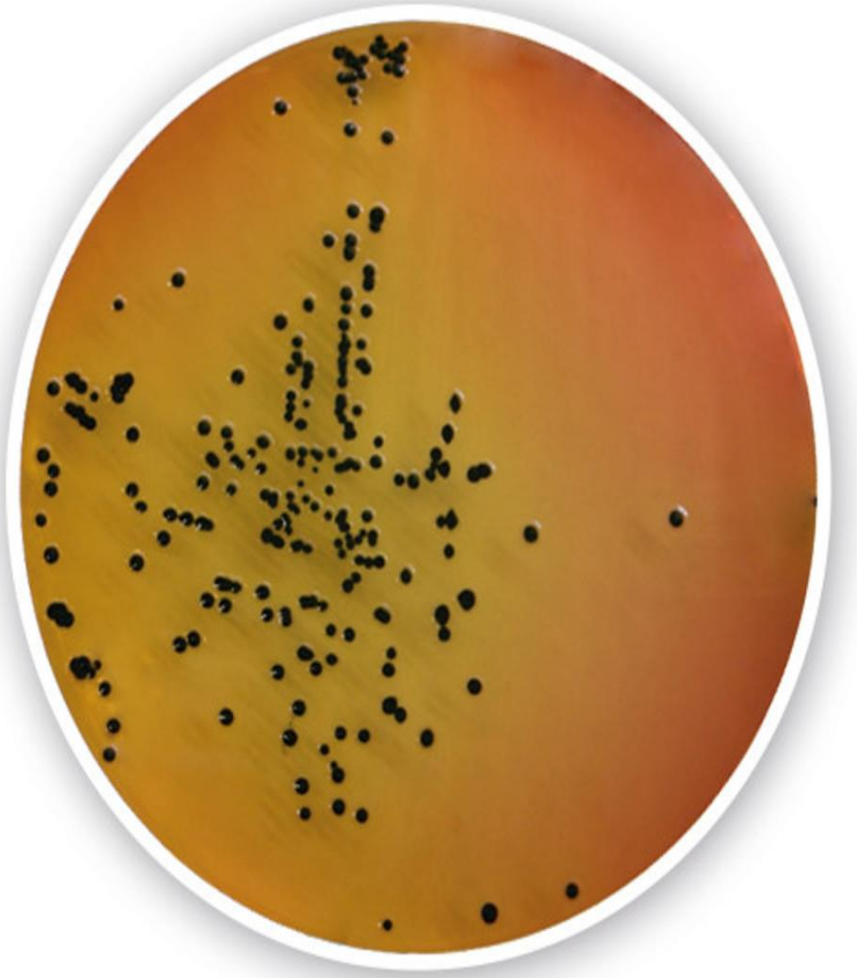
- Aerobic / Facultatively anaerobic
- Grows on simple media - Nutrient agar,
- Temp 15 - 41°c / 37° c
- Colonies appear as large 2 -3 mm, circular, low convex,
- On MacConkey medium appear

  Colorless ( NLF )

Selective Medium - Wilson Blair Bismuth sulphide medium. Produce Jet black colonies

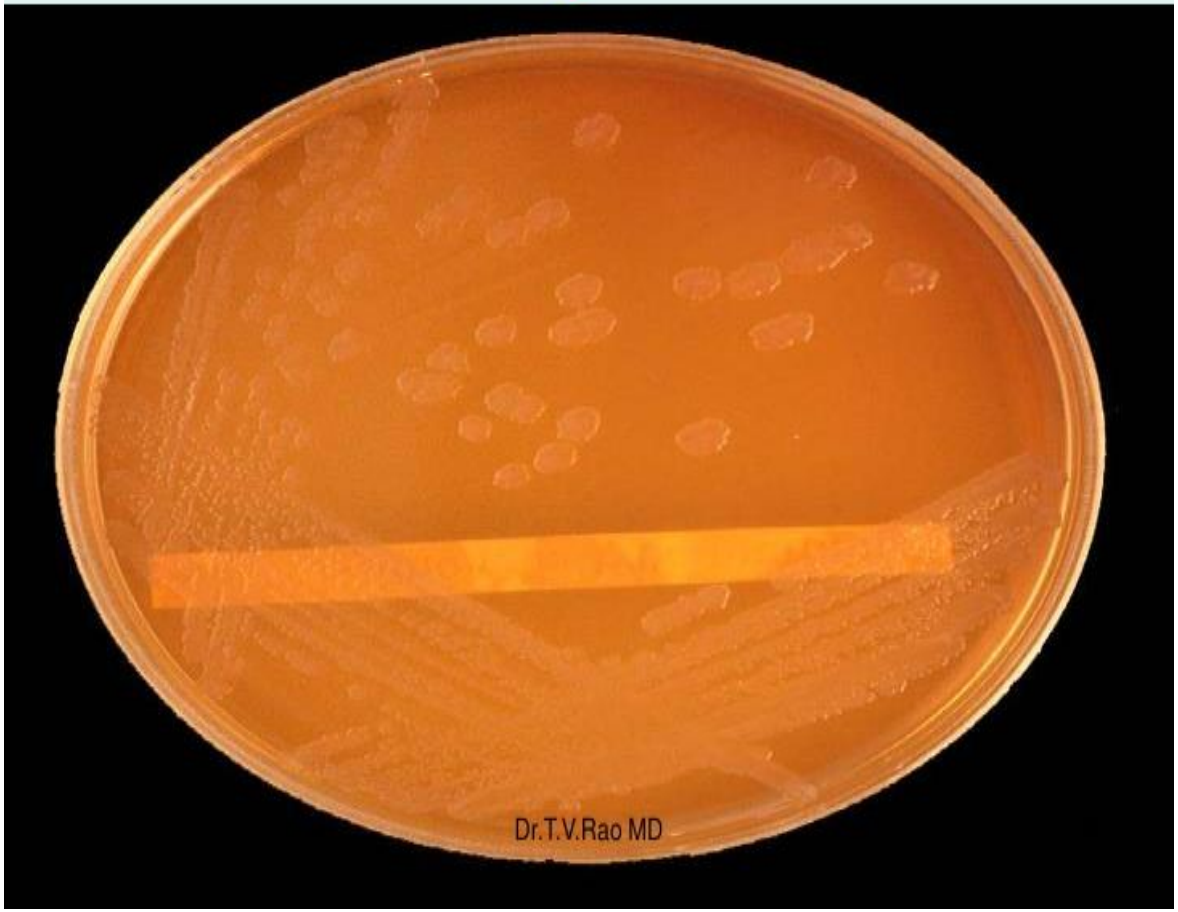
$H_2S$ produced by Salmonella typhi

***Salmonella spp.***
***SS (salmonella-shigella) agar***



***Salmonella spp.***
***Bismuth Sulfite agar***

# Salmonella on Mac Conkey's agar



# Cultural character

- Grow on ordinary culture media
- In MacConkey agar and DCA: Small, circular, translucent, nonlactose fermenting colonies.
- In Wilson and Blair Bismuth sulfite medium: Black colonies with metallic sheen due to production of $H_2S$
- Selenite F and tetrathionate broth (enrichment media for stool specimen culture)

# Biochemical Characters

- Glucose ,Mannitol ,Maltose produce A/G
- Salmonella typhi do not produce gas
- Lactose/Salicin/sucrose not fermented.
- Indole -
- Methyl Red +
- V P -
- Citrate +
- Urea -
- $H_2S$ - produced by Salmonella typhi
- Paratyphi A do not produce $H_2S$

- Salmonella can cause

3. Enteric fever
4. Gastroenteritis
5. Septicemia with or without focal suppuration
6. Carrier state

# Pathogenicity

- Enteric Fever-
2. Typhoid fever- S. typhi
3. Paratyphoid fever – S.paratyphi A,B,C

- Salmonella typhi—typhoid fever
- Eberth-Gaffky bacillus/Eberthella typhi

- Practically salmonella has been divided into

6. The enteric fever group-typhoid,paratyphoid bacilli
7. The food poisoning group

# Clinical Manifestations

- Typhoidal salmonella – Enteric fever
- Non typhoidal salmonella – Gastroenteritis

- Bacteremia
- Osteomyelitis
- Localised infections
- Carriers

# Clinical manifestations

- Head ache, malise,anorexia ,coated tongue
- Abdominal discomfort,
- Constipation / Diarrhea
- Step ladder type fever,
- Relative bradycardia,
- A soft palpable spleen
- Hepatomegaly
- Rose spots appear

# PATHOPHYSIOLOGY

Ingest contaminated food

↓

Ingested bacilli invade small intestinal mucosa

↓

Taken up by macrophage & transported to regional lymph node

↓

S.typhi multiply in the intestinal lymphoid tissue

↓

Intact with enterocytes & M cells(ileal Peyer's pathches) during the 1-3 week of incubation period ( Diarrhoea )

↓

End of incubation period, bacilli enter bloodstream(Bacteraemia phase)

↓ ( onset of typhoid fever )

Bacteria invade the gallbladder, biliary system and lymphatic tissue of the bowel and multiply in high number

↓

Then pass into the intestinal tract (stool)

# CLINICAL FEATURES

## Stage 1 (1ST WEEK)

- Slowly rising (stepladder fashion) of temperature for 4-5 days
- Abdominal pain & myalgia
- Malaise
- Headache
- Constipation
- Relative bradycardia

## Stage 2 (2ND WEEK)

- Signs and symptoms of 1st week progress

## End of 2ND WEEK

- Delirium, complications, then coma & death (if untreated)

## End of 1ST WEEK

- Rose spots may appear on the upper abdomen & on the back of sparse
- Cough
- Splenomegaly
- Abdominal distension with tenderness
- Diarrhea

# Laboratory diagnosis of Enteric fever

- Typhoid fever + Paratyphoid fever

- Typhoid fever – S.Typhi
- Paratyphoid fever – S.Paratyphi A, B, and C

# Specimen collection

Blood
Serum
Urine
Feces
BoneMarrow
Bile

Pus
CSF
Sputum
Gall bladder
Liver
Spleen
Mesentric lymph nodes

# Ideal specimen

| | |
|---|---|
| First week | Blood (culture) |
| Second week | Serum (Antibodies) |
| Third week | Stool |
| Fourth week | Urine |

# Chance of isolation

| Specimens | First week | Third week |
|---|---|---|
| Blood | 50 to 80% | 30% |
| Feces | 40 to 50% | 80% |
| Urine | - | 25% |

# Blood culture

- Volume of blood :

  10 to 15 ml from adults and adolescents , 2 to 4 ml in children
- Ratio of blood to bile broth: 1:10
- Or add saponin to BHI broth with 0.05% SPS
- Inoculate the blood immediately
- Transport immediately, never store under 15degC
- Incubate as soon as possible

# Diagnosis of Enteric Fever

## *Widal test*

- Serum agglutinins raise abruptly during the 2nd or 3rd week
- The Widal test detects antibodies against O and H antigens
- Two serum specimens obtained at intervals of 7 - 10 days to read the raise of antibodies.
- Serial dilutions on unknown sera are tested against the antigens for respective Salmonella
- False positives and False negative limits the utility of the test
- The interpretative criteria when single serum specimens are tested vary
- Cross reactions limits the specificity

# Widal Test

- Single test not diagnostic.
- Paired samples tests
- Diagnostic.
  - O > 1 in 80
  - H > 1in 160

H agglutinins appear first

False positives in
- Unapparent infection,
- Immunization
- Previously infected

# Antimicrobial susceptibility testing

Drugs :

1. Amoxycillin
2. Co-amoxiclav
3. Cefuroxime
4. Cotrimoxazole
5. Ciprofloxacin
6. Chloramphenicol

# Vaccines

- An Injectable vaccine Typhium Vi
- Contains purified Vi polysaccharide antigen from S.typhi strain Ty2
- A single dose, subcutaneous route
- Given to children > 5 years
- Immunity lasts for 2- 3 years.
- Follow a booster

# Salmonellosis

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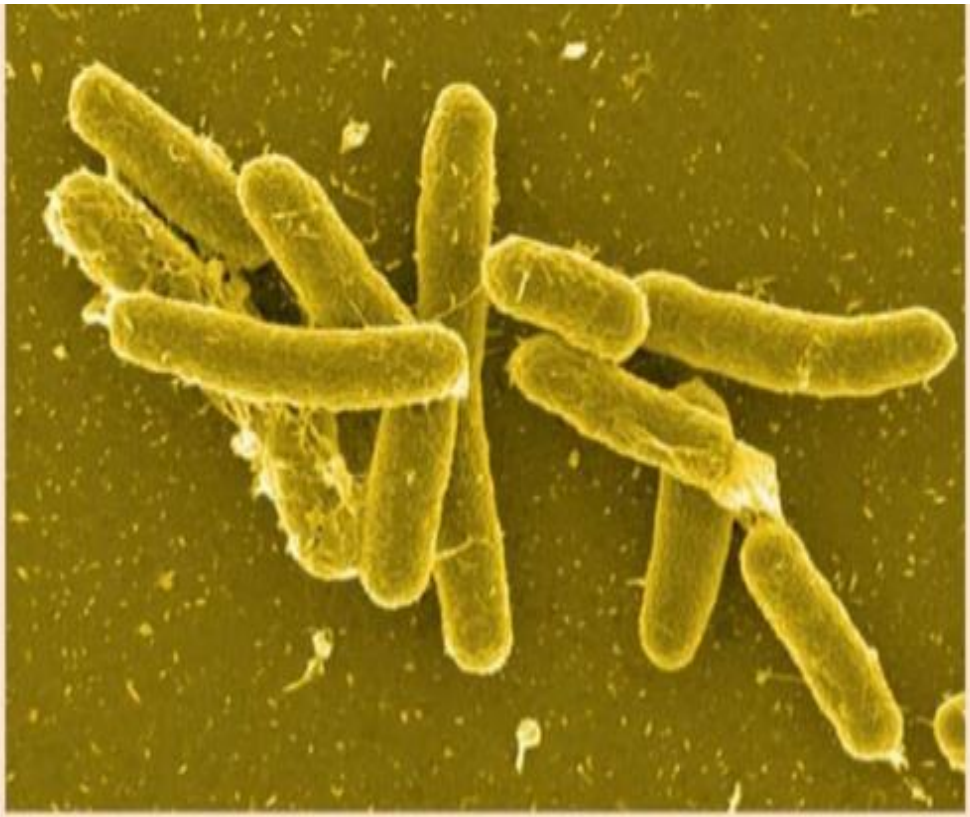
Padmashree School of Public Health Bangalore.
(Affiliated by Rajiv Gandhi University of Health Sciences, Bangalore)



- Gram Negative Bacteria
- Species:
  - S. Bongori
  - S. Enterica
  - Six sub species
- More than 2500 Serovars/Serotypes
- Many are zoonotic

# Human Transmission

- Fecal-oral: direct or indirect
- Commonly contaminated items
  - Meat, eggs, water
- Fecal material from:
  - Reptiles
  - Chicks
  - Ducklings
  - Livestock, dogs, cats, adult poultry

# Pathophysiology

- Bacteria colonizes in the ilium – the end part of small intestine
- They invade intestinal epithelium
- They expand within the epithelium and lymphoid cavities
- The invasion happens when organisms start to ruffle
- This way they stimulate the pinocytosis


- They multiply & spread through mesenteric lymph nodes
- After reaching intestines they create an acute inflammation

## CLINICAL PRESENTATION

Incubation period: 6-72 hours

but illness usually occurs within 12-36 hours after exposure

Symptoms:

- acute diarrhea
- abdominal pain
- fever
- Vomiting
- Headache, fever, chills, myalgia
- Severe dehydration: infants, elderly

- The illness usually lasts 4-7 days, and most people recover without treatment.

- Rates of invasive infections and death are generally higher among infants, older adults, and people with immunosuppressive conditions (including HIV), hemoglobinopathies, and malignant neoplasms.

# DIAGNOSIS

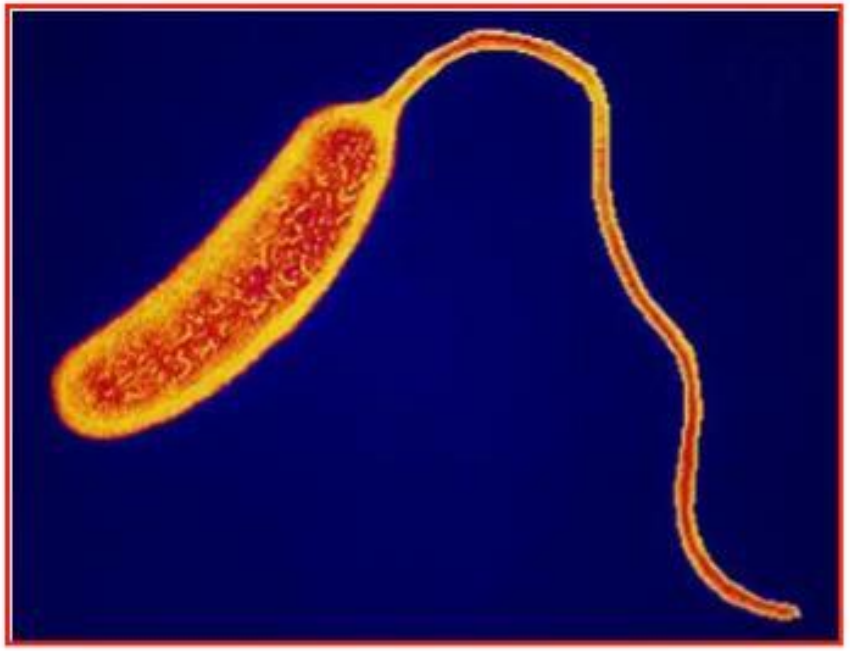
- Diagnosis is based on isolation of Salmonella organisms
- About 90% of isolates are obtained from routine stool culture, but isolates are also obtained from blood, urine, and material from sites of infection
- Isolates of salmonellae are needed for serotyping and antimicrobial susceptibility testing
- PCR

# Antibiotic Therapy for Nontyphoidal Salmonella Infection in Adults

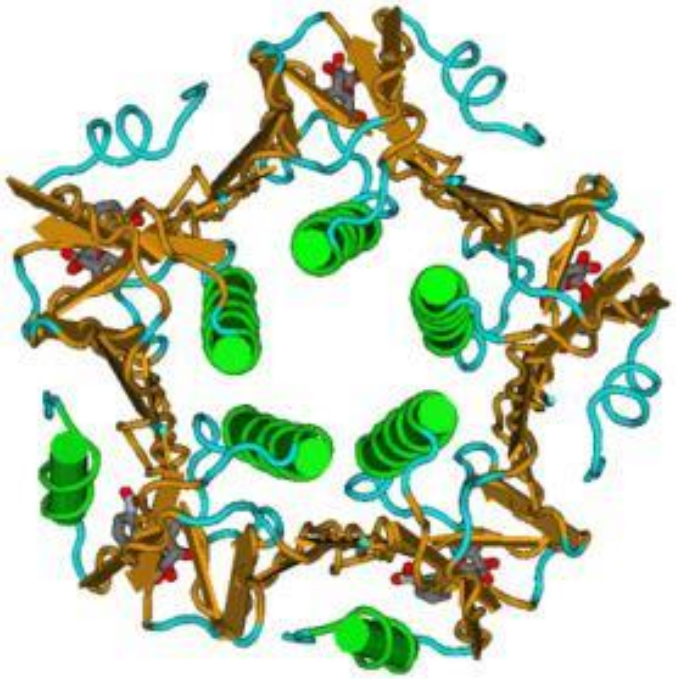
| Indication | Agent | Dosage (route) | Duration, days |
|---|---|---|---|
| **Preemptive Treatment** | | | |
| | Ciprofloxacin | 500 mg bid (PO) | 2–3 |
| **Severe Gastroenteritis** | | | |
| | Ciprofloxacin | 500 mg bid (PO) or 400 mg q12h (IV) | 3–7 |
| | Trimethoprim-sulfamethoxazole | 160/800 mg bid (PO) | |
| | Amoxicillin | 1 g tid (PO) | |
| | Ceftriaxone | 1–2 g/d (IV) | |

# Vibrio Cholerae

- Gram negative
- Facultative anaerobe
- Curved-shaped rod
- One polar flagellum
- Housed by zooplankton in both fresh and salt water
- Found in water contaminated with fecal matter
- Cholera Toxin



# Cholera Toxin



- A-B Exotoxin
- Causes Cholera in humans
- Produced by the CTXf bacteriophage
- A subunit (activating)
  - A1 domain: enzymatic active site
  - A2 domain: a-helical tail
- B subunit (binding)
  - Pentameric ring around central pore

# Bio Chemical Reactions

| | V.cholrae( Classical ) | V.cholrae (El Tor) |
|---|---|---|
| Hemolysis | -ve | +ve |
| Voges -proskauer test | -ve | +ve |
| Polymyxin sensitivity | +ve | -ve |
| Group IV phage Susceptibility | +ve | -ve |
| Chick erythrocyte Agglutination | -ve | +ve |

# Diagnosis

- Stool culture: Toxigenic *Vibrio cholerae* O1
- Use Cary Blair Transport media if available
  - Viable for many days at room temperature
- Use TCBS media for culture
- Use *V. cholerae* serogroup O1 antisera
- Confirm presence of cholera toxin
- Cholera Rapid Test Dipsticks

# How is the Infection Diagnosed?

- Isolated from stool or vomit
- Cholera antibodies in the blood
- Fecal leukocyte stain
- Stool culture
- Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar

# Selective Medium - TCBS

- V.cholrae grows well on Thiosulphate citrate bile sucrose (TCBS ) agar, on which it produces yellow colonies that are readily visible against the dark green background of the agar.

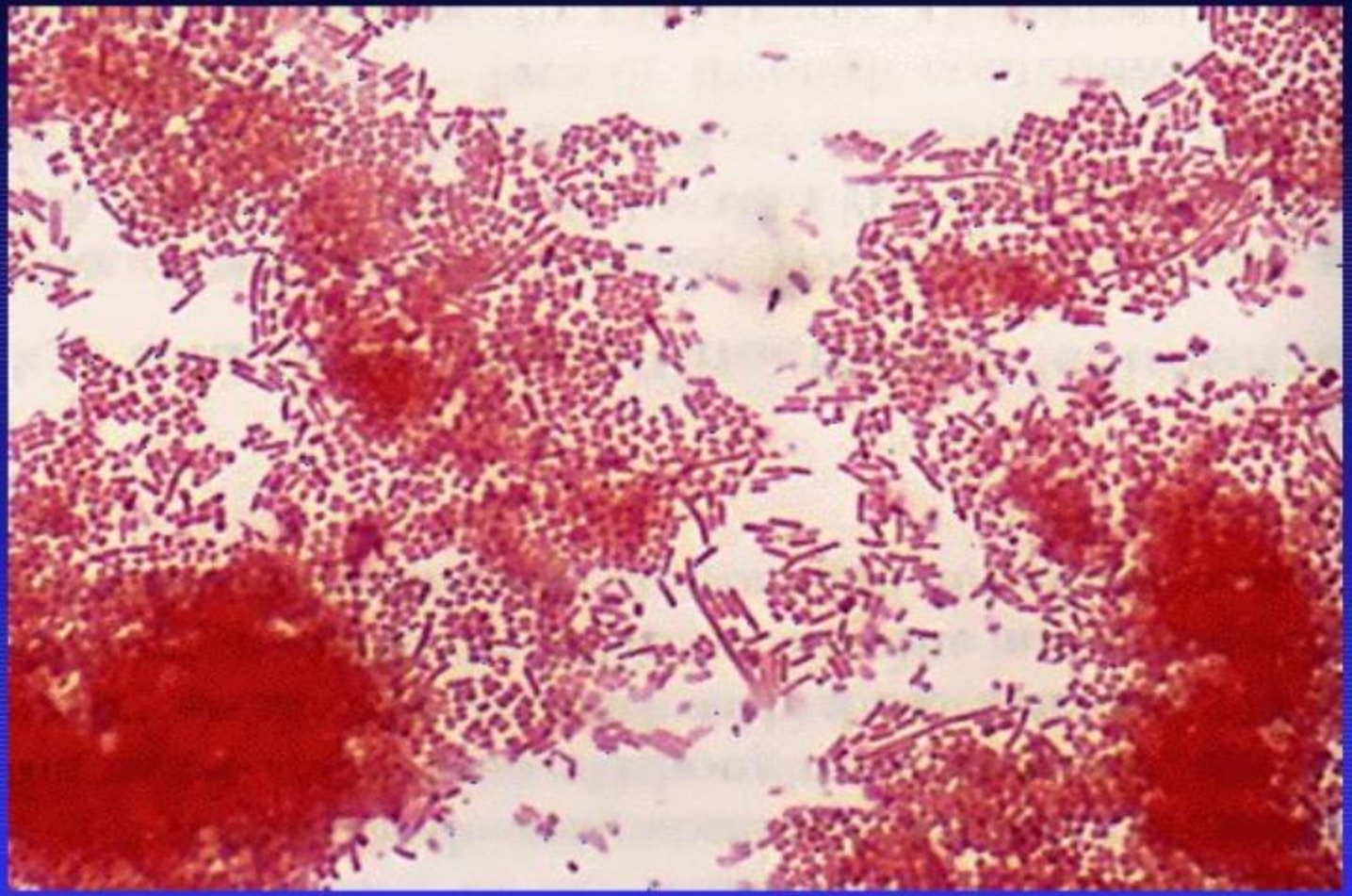
# Clinical manifestations

- Diarrhea occurs as much as 20 – 30 Liters/Day fluids are lost.
- Results in dehydration
- Shock
- Acidosis
- Can lead to death.
- About 60% of infections are caused with classic V.cholrae and are asymptomatic, about 75% of infections are caused by

# Bacteroides

- *B. fragilis* is most significant.
- Pale irregularly staining Gram negative bacilli/ coccobacilli with polysaccharide capsule.
  - Pleomorphic
- Normal flora of large intestine & female genital tract. Normal stool contain $11^{10}$ *B. fragilis organisms per gram.*
- Cause abdominal, lung and brain abscesses and wound infection

# Bacteroides



# *Bacteroides* Virulence Factors

- Polysaccharide capsule
- Lipopolysaccharide
- Agglutinin
- Histolytic enzyme
- Oxygen tolerance
- β lactamase

# Genus Clostridium

- In Anaerobic spore bearing Gram positive bacilli Spores are wider than the body giving spindle shape
- The name derived from word **Kolster meaning spindle**

# Clostridium

***C. perfringens*: gas gangrene; food poisoning**

***C. tetani*: tetanus**

***C. botulinum*: botulism**

***C. difficile*: pseudomembranous colitis**

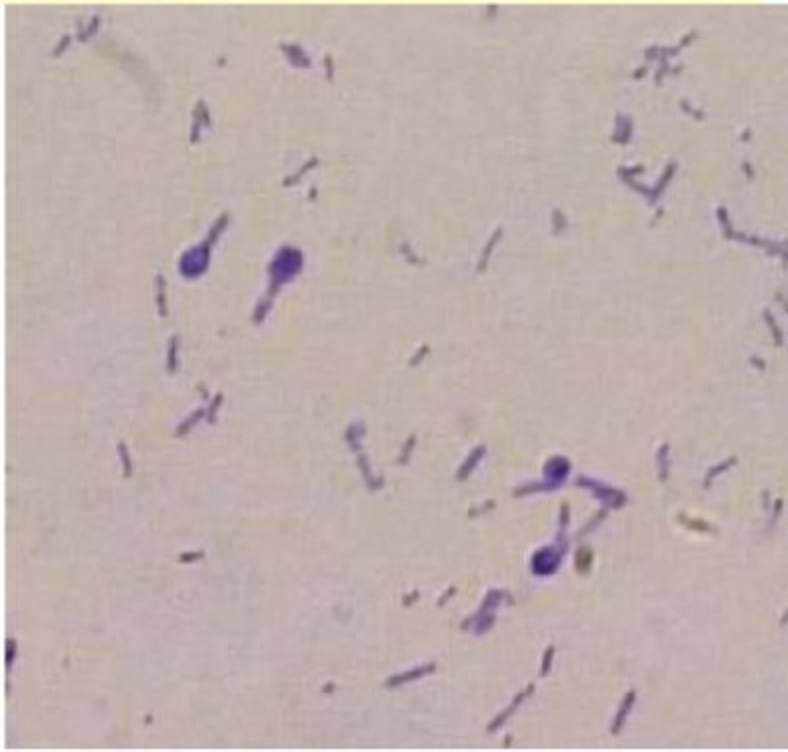
## Physiology and Structure

**Anaerobic.**

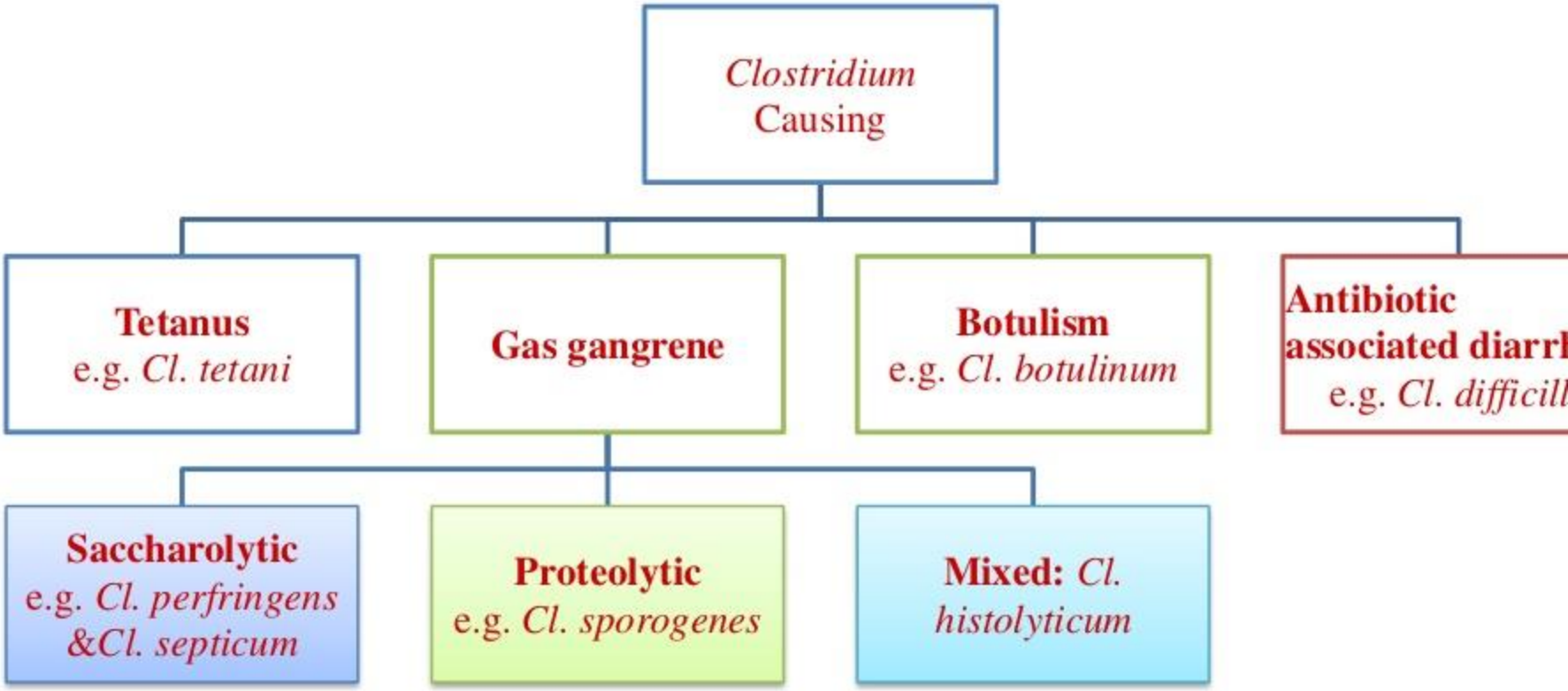
Large gram-positive rods.

The spores are usually wider than the rods, and are located terminally or sub terminally.

Most clostridia are motile by peritrichous flagella.

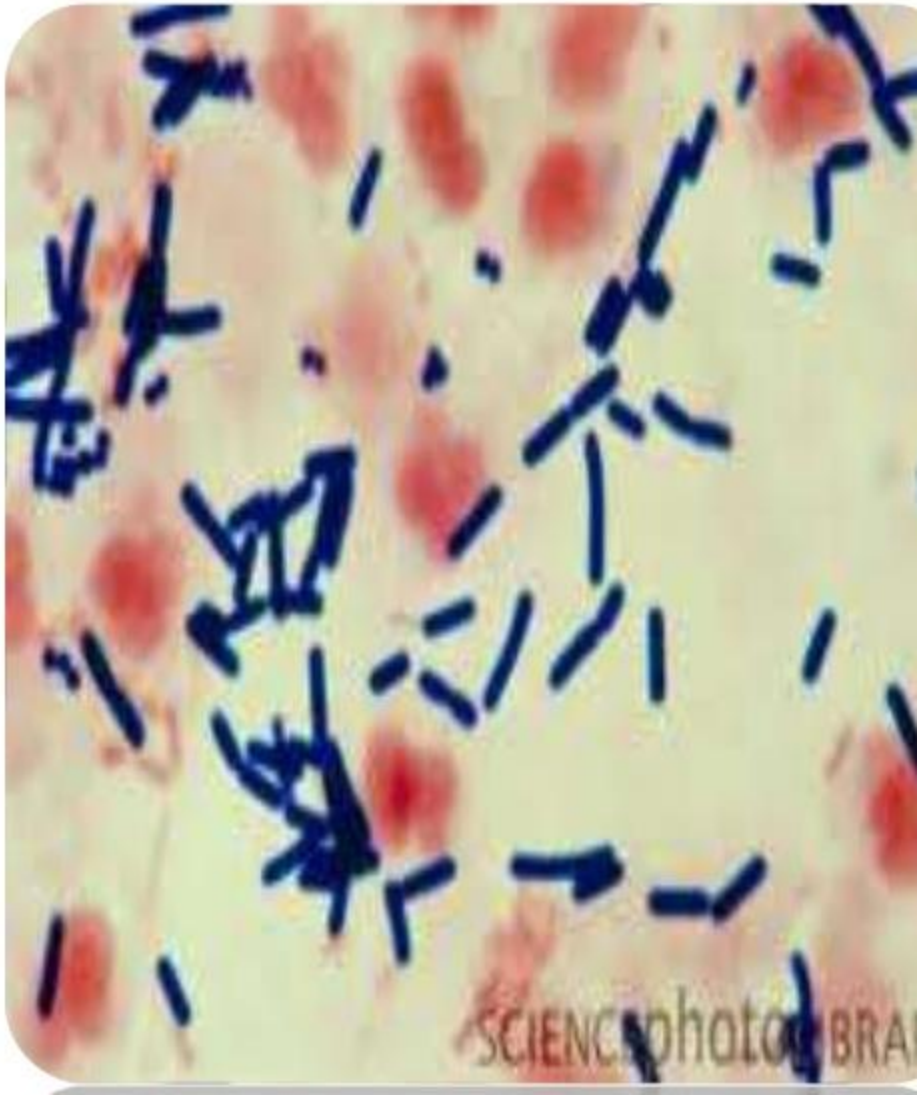


# Clostridia of medical importanc



# How they appear in Gram staining

- They are Gram positive, but may appear to be Gram negative. All produce spores, which enable the organisms to survive in adverse conditions, for example in soil and dust and on skin.



# *Clostridium perfringens*

- Large Gram-positive bacilli with stubby ends
- **Capsulated**
- **Non motile** Anaerobic
- Grown quickly on selective media
- **Can be identified by** Nagler reaction

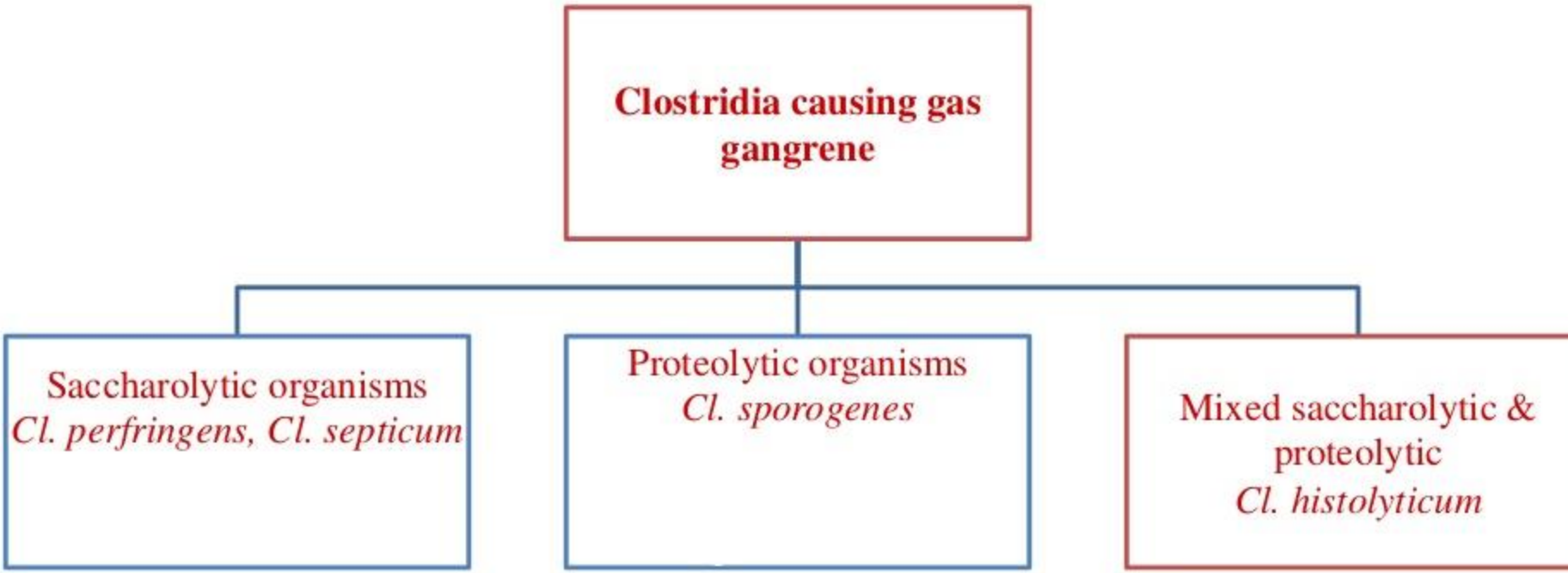
# Some Clostridia Produce Gas gangrene

- The organisms associated **with gas gangrene** attack soft tissues by producing toxins and aggressins, and are referred to as histotoxic. C. difficile and some strains of **C. perfringens** produce enterotoxins.

# C. perfringens

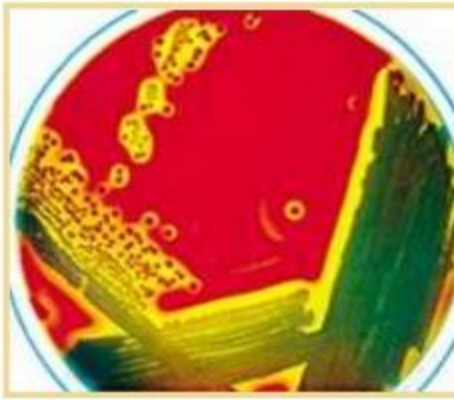
- C. perfringens is a relatively large Gram-positive bacillus (about 4-6 × 1 μm) with blunt ends. It is capsulate and non-motile. It grows quickly on laboratory media, particularly at high temperatures (approximately 42°C), when the doubling time can be as short as 8 min. I

# Clostridium Causing Gas Gangren



# The Agent

- **Clostridium perfringens**
  - Gram-positive bacteria
    - Anaerobic rod
    - 3-8 x 0.4 – 1.2 mu
  - Found in soil, decaying matter and intestinal tract of mammals
- 5 types (A-E)
  - Types B and D produce the epsilon toxin



# Micro & Macroscopic C. perfringen

**NOTE:** Large rectangular gram-positive bacilli

**NOTE:** Double zone of hemoly

Inner beta-hemolysis = θ to

# Resistance

- Vegetative bacteria is killed like other bacteria
- Cl.perfringens destroyed by boiling
- Cl, botulinum not killed even at 105 c 0 for less than 100 minutes
- All spores are killed at 121 oc in 20 minutes
- Halogens , Glutaraldehyde are effective on spores
- Metronidazole and Pencillin and Chloramphenicol are effective

# How Clostridia are Cultivated

- Clostridia grow well on ordinary medium under anaerobic medium

# Media used for Cultivation

- Liquid medium for cultivation  cooked meat broth
- Thiglyclolate broth
- CMB contain unsaturated fatty acids which take up oxygen
- Proteolytic medium turns the medium black and Saccharolytic medium turn the meat pink

# Virulence Factors

- Virulence factors
  - –toxins –
    - alpha toxin – causes RBC rupture, edema and tissue destruction
  - –collagenase
  - –Hyaluronidase
  - –DNase

# Toxins

- **The toxins of *Cl. perfringens***
  - **α toxin** (phospholipase C, lecithinase) is the most important toxin
    - Lyses of RBCs, platelets, leucocytes and endothelial cells
    - Increased vascular permeability with massive hemolysis and bleeding tissue destruction
    - Hepatic toxicity and myocardial dysfunction
  - **β-toxin** is responsible for necrotic lesions in necrotizing enterocolitis
  - **Enterotoxin** is heat labile toxin produced in colon → food poisoning

# C. perfringens

## Clinical Diseases

### Gas gangrene

Spores germinate vegetative cells multiply, ferment carbohydrates and produce gas in the tissue. This results in distension of tissue and interference with blood supply the bacteria produce necrotizing toxin and Hyaluronidase, which favor the spread of infection tissue necrosis extends, resulting in increased bacterial growth, hemolytic anemia, then severe toxemia and death.

Incubation: 1-7 days after infection.

**Symptoms:** Crepitation in the subcutaneous tissue and muscle, foul smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, renal failure, and death.

Can be also caused by other *Clostridium* species.

# Clostridial Cellulitis

# C. perfringens

## Laboratory Diagnosis

Specimens: pus, necrotic tissue, feces, food, etc.

Smears: large gram-positive rods with or without spores, usually in the absence of leukocytes.

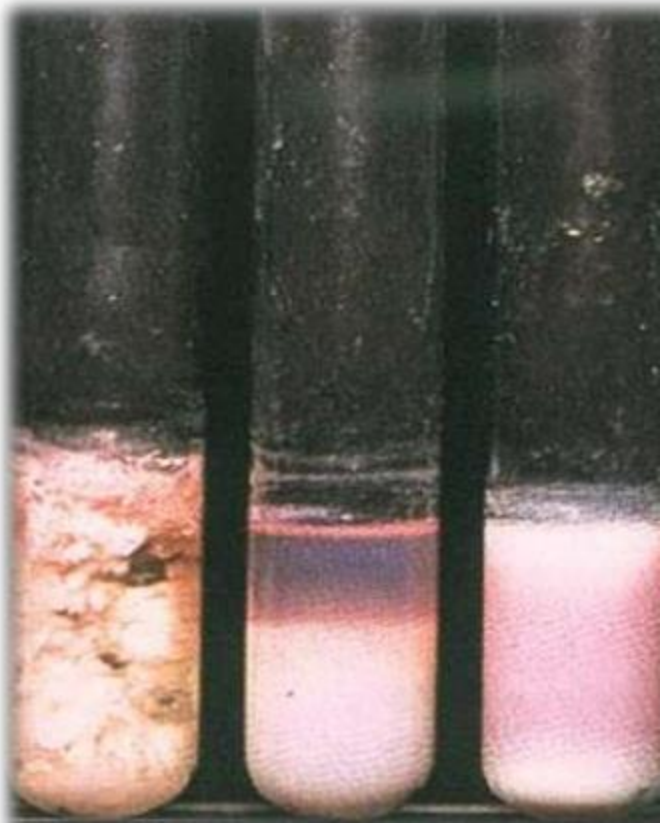
Culture: anaerobic culture on blood plate.

Identification:

"Storming fermentation"-- clot torn by gas in 24 hrs.

Lecithinase test-- precipitate formed around colonies on egg yolk media.

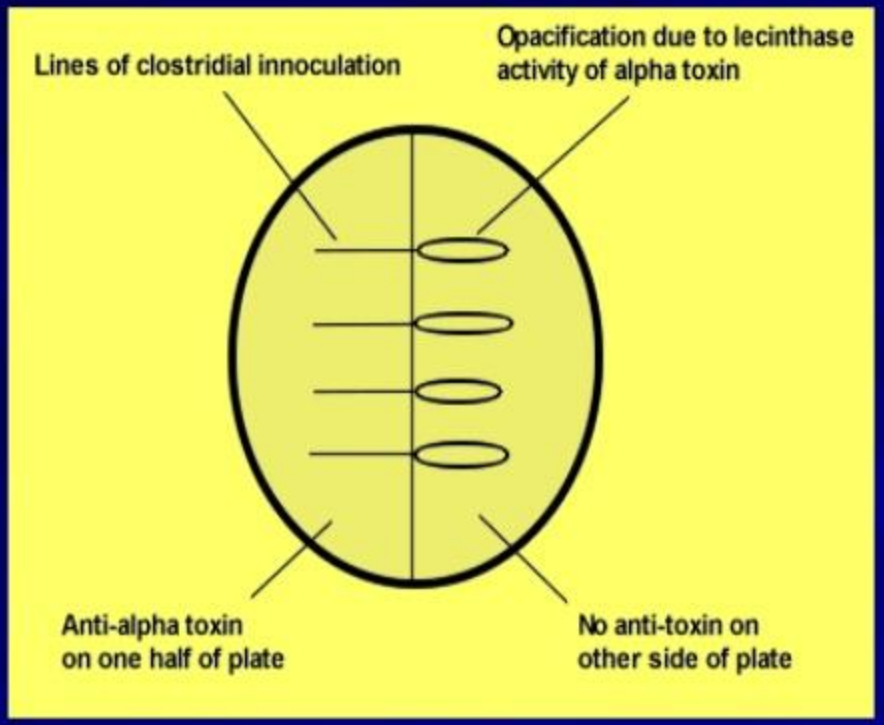
Biochemical tests.



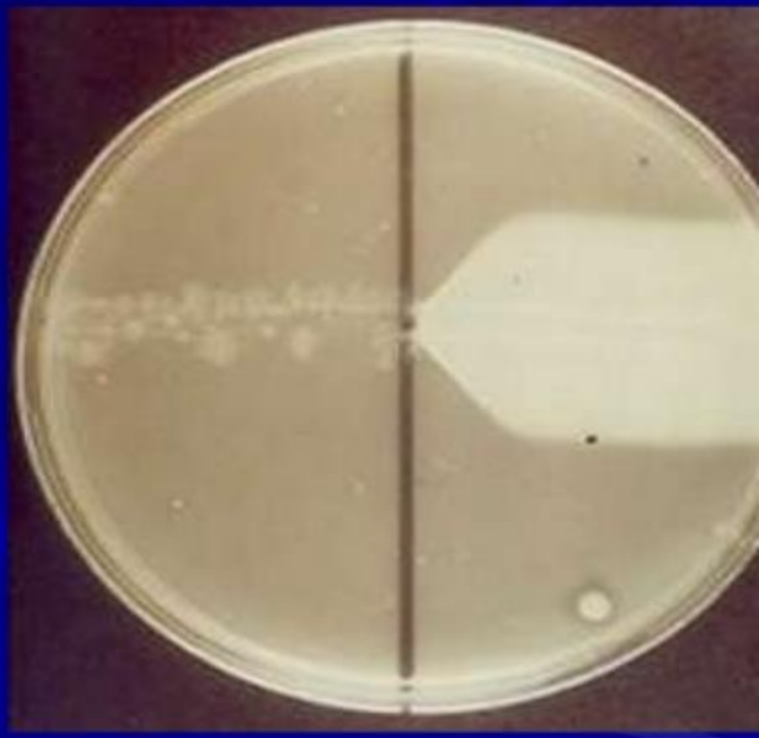
# *C.* perfringens Nagler Reaction

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

# Nagler Reaction




Procedure of Nagler Reaction



Positive Nagler Reaction

# Biochemical Tests

- *Cl. perfringnes* characterized by:
  - ➢ It ferments many carbohydrates with acid & gas
  - ➢ It acidified litmus milk with stormy clot production
  - ➢ Nagler reaction is positive

# *Clostridium tetani*

Anaerobic bacteria of the genus species *Clostridium* it is gram positive, slender bacillus and it has spherical terminal spores giving drum stick appearance

It is non capsulated & motile with peritrichus flagella

*It* produces a potent biological toxin, tetanospasmin, and is the causative agent of tetanus a disease characterized by painful muscular spasms that can lead to respiratory failure and, in up to 40% of cases, death.

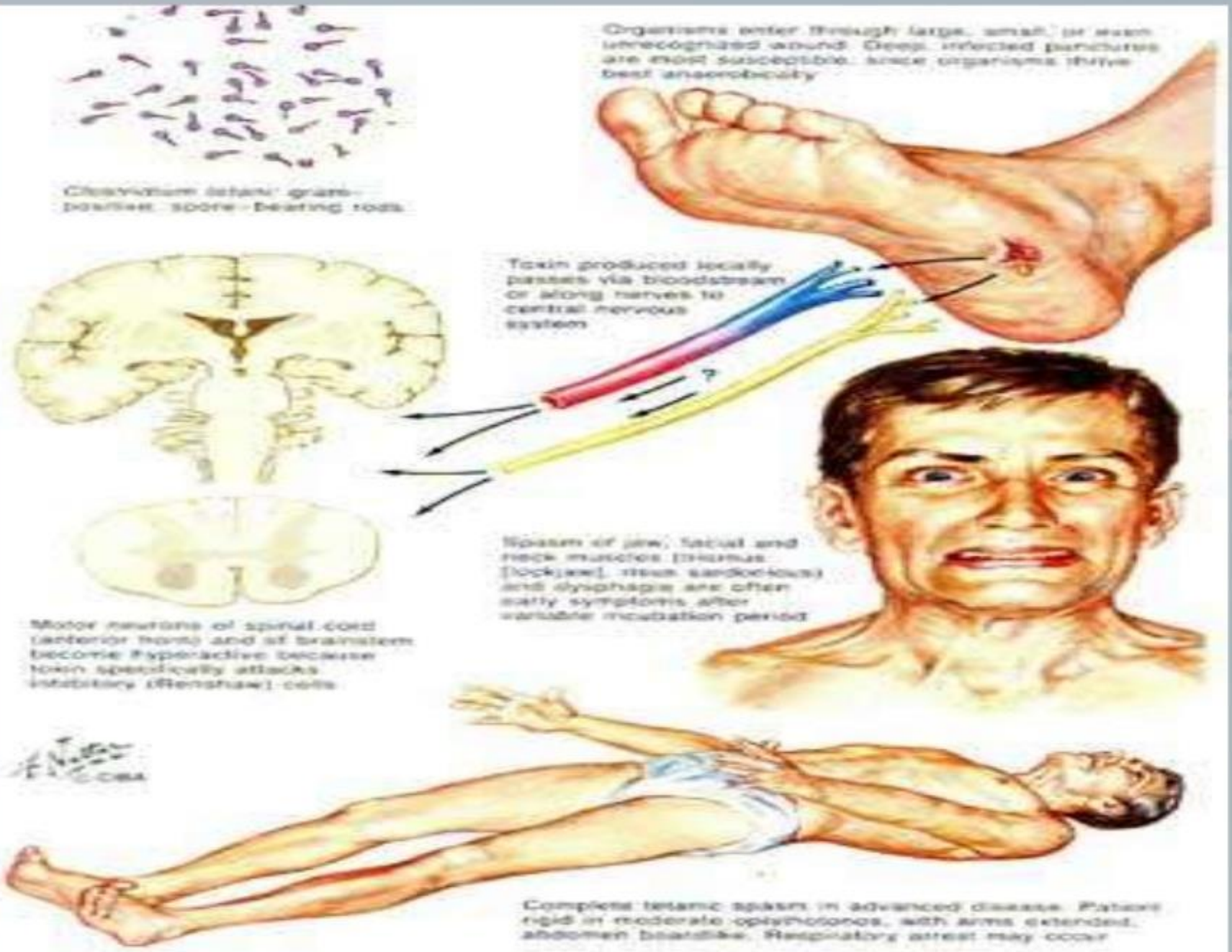
# Cl. Tetani

- Soil and GIT
- Terminal spores – drumstick appearance
- Non capsulated, motile except type VI
- Grows on BA,NA,CMB, Thioglycoslate broth
- CMB- Black, BA – Swarming,
- Iridescence – greenish fluorescence on MA
- No sugars fermented

# What is Tetanus?

- An infectious disease caused by contamination of wounds from the bacteria ***Clostridium tetani***, or the spores they produce that live in the soil, and animal feces

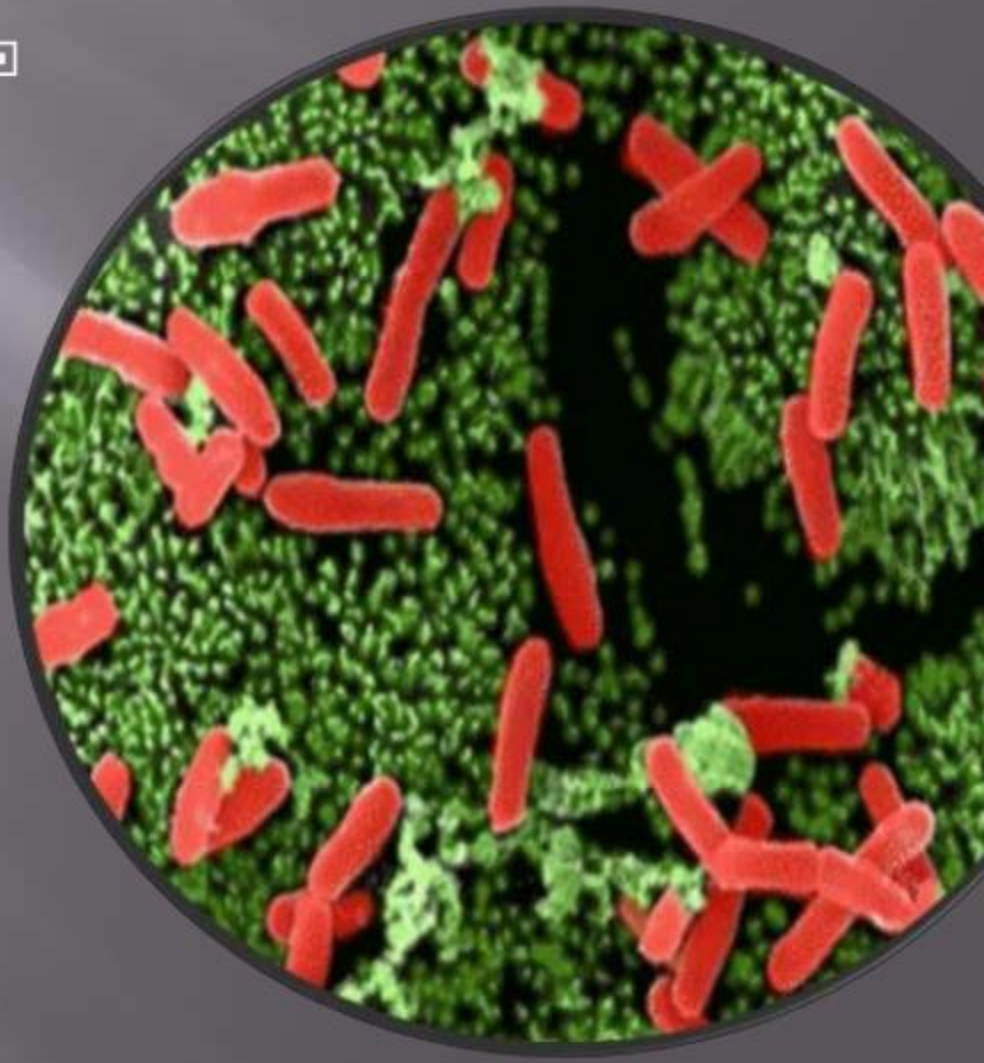
- Infection follows **when spores**
- **become activated and develop**
- into gram-positive bacteria that multiply
- and produce a very powerful toxin (**tetanospasmin**) that affects the muscles.


Clostridium tetani: gram-positive, spore-bearing rods
Organisms enter through large, small, or even unrecognized wound. Deep, infected punctures are most susceptible, since organisms thrive best anaerobically
Toxin produced locally passes via bloodstream or along nerves to central nervous system
Spasm of jaw, facial and neck muscles (trismus [lockjaw], risus sardonicus) and dysphagia are often early symptoms after variable incubation period
Motor neurons of spinal cord (anterior horns) and of brainstem become hyperactive because toxin specifically attacks inhibitory (Renshaw) cells
Complete tetanic spasm in advanced disease. Patient rigid in moderate opisthotonos, with arms extended, abdomen boardlike. Respiratory arrest may occur

# Clostridium difficile

# *Clostridium difficile*

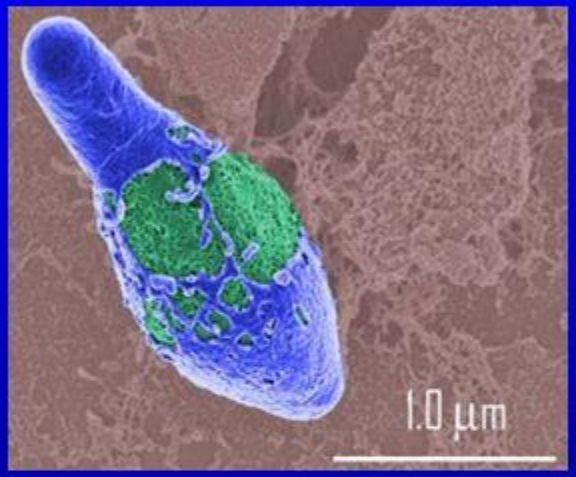
- "C. diff", is a species of Gram-positive bacteria of the genus *Clostridium* that causes diarrhea and other intestinal disease when competing bacteria are wiped out by antibiotics.



# *Cl. Botulinum*

- Botulus = sausages
- Soil, manure, vegetables
- Strict aerobes grow on ordinary media
- Large fimbriate irregular colonies
- GPB with sub-terminal highly resistant spores
- Classification based on toxin produced – 8 types A (most toxic), B, C 1- 3, D, E, F, G

# Bacterium of the day: *Clostridium botulinum*




**CLOSTRIDIUM BOTULINUM**

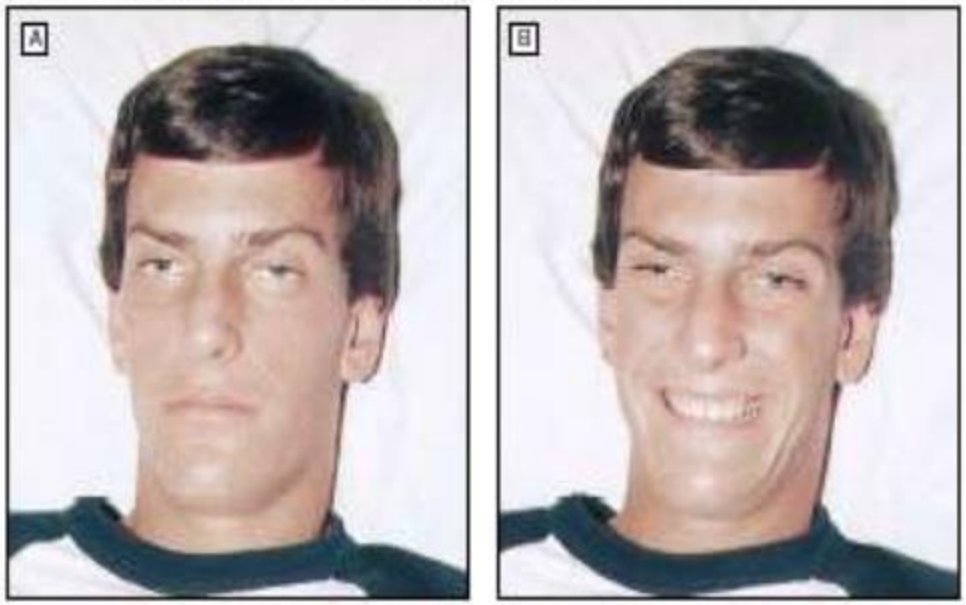
**Clostridium botulinum is an anaerobic, Gram-positive, spore-forming rod that produces a potent neurotoxin. The spores are heat-resistant and can survive in foods that are incorrectly or minimally processed.**

**Foodborne botulism is a severe type of food poisoning caused by the ingestion of foods containing the potent neurotoxin formed during growth of the organism. The toxin is heat labile and can be destroyed if heated at 80°C for 10 minutes or longer .The disease is of considerable concern because of its high mortality rate if not treated immediately and properly. It can happen with inadequately processed, home-canned foods, in meat products, sausages, canned vegetables and seafood productc.**

# Signs of Food-borne and Wound Botulism

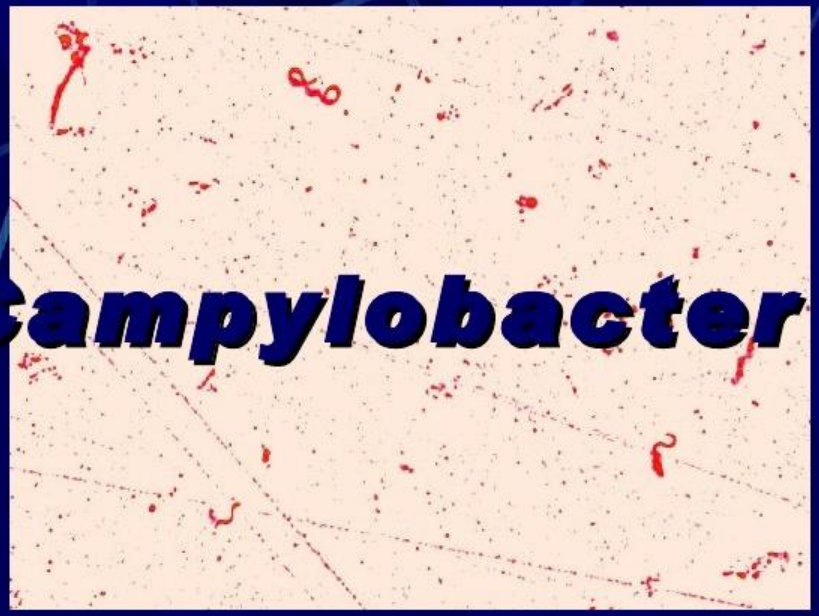
- **Ventilatory (respiratory) problems**
- **Eye muscle paresis/paralysis (extra ocular, eyelid)**
- **Dry mucous membranes in mouth/throat**
- **Dilated, fixed pupils**
- **Ataxia**
- **Hypotension**
- **Nystagmus**
- **Decreased to absent deep tendon reflexes**

**Figure 2.** Seventeen-Year-Old Patient With Mild Botulism




A. Patient at rest. Note bilateral mild ptosis, dilated pupils, disconjugate gaze, and symmetric facial muscles.
B, Patient was requested to perform his maximum smile. Note absent smile creases, ptosis, minimally asymmetric smile.

# Campylobacter



# *Campylobacter*

- Among the most widespread cause of infection in the world.
- Cause both diarrheal and systemic diseases
- *Campylobacter jejuni*

# Typical Organisms

- Gram-negative rods with comma, S, or “gull-wing” shapes.
- Motive, with a single polar flagellum
- No spore & no capsule

# Culture

- An atmosphere with reduced $O_2$ (5% $O_2$) with added $CO_2$ (10% $CO_2$)
- At 42 ℃ (for selection)
- Several selective media can be used (eg, Skirrow's medium)
- Two types of colonies:
  - ☺ watery and spreading
  - ☺ round and convex

# Virulence Factor

- Lipopolysaccharides (LPS) with endotoxic activity
- Cytopathic extracellular toxins and enterotoxins have been found

# Pathogenesis

- The infection by oral route from food, drink, or contact with infected animals or animal products(**Milk, meat products** ).
- Susceptible to gastric acid (about $10^4$ organisums)

# *Campylobacter* - symptoms

- Incubation: 4-8d
- Acute enteritis: 1w, stools remain positive for 3 w
- Acute colitis
- Acute abdominal pain
- Bacteremia: <1% *C. jejuni*
- Septic abortion
- Reactive arthritis

- diarrhea
- malaise
- fever
- abdominal pain
- usually self-limiting
- antibiotics occassionally
- bacteremia
  - –small minority

# Diagnostic Laboratory Tests

- Specimens: Diarrheal stools
- Smears: Gram-stained smears of stool may show the typical “gull-shaped” rods.
- Culture: (have been described above)

# *Helicobacter pylori*

- Curved bacilli –
- Former name - *Campylobacter pylori*,
- ***H. pylori***

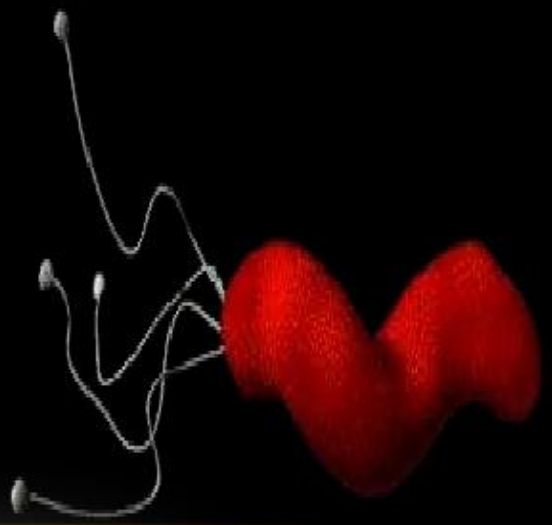

# WARREN AND MARSHAL WINS NOBEL PRIZE

# *Helicobacter pylori*

- *Helicobacter pylori* is the prototype organism in this group. It is associated with antral gastritis, gastric ulcers, and gastric carcinoma.

# HELICOBACTER PYLORI

- Gram –ve spiral shaped , motile with unipolar tuft of lopotrichus flagella



# *H. PYLORI* BACTERIA

- Gram negative
- Spiral rod
- Unipolar flagella
- Microaerophilic
- Urease positive*

*Most important character

*Scanning microscopic view of *H. pylori*

# CULTURING AND BIOCHEMICAL CHARACTERS

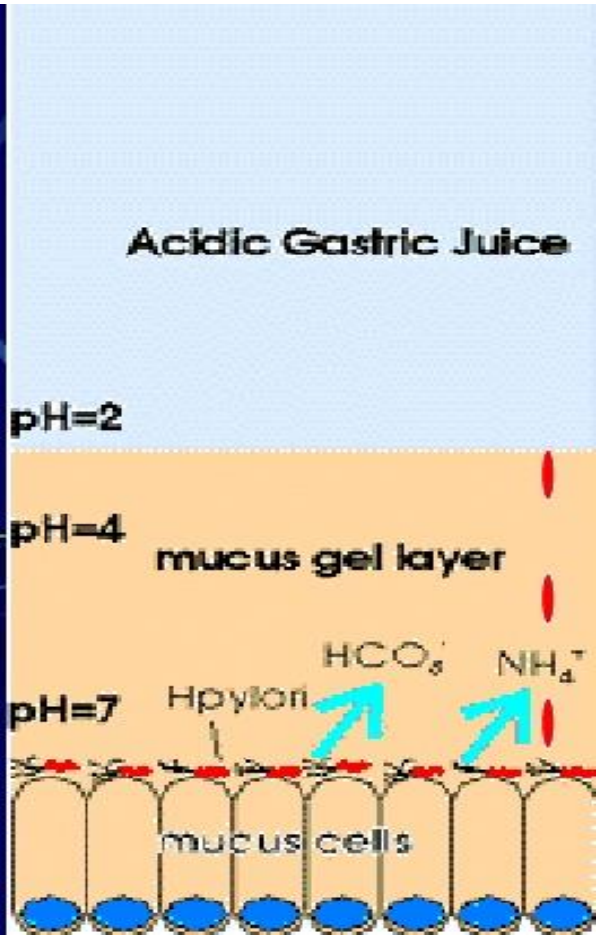
- Grows on chocolate agar, Campylobacter media
- Grows under Microaerophilic conditions
- With presence of 5 – 20% co2
- Oxidase +
- Catalase –
- Urease strongly +++
- H2S

# Virulence factors

- vacA (vacuolationg associated) cytotoxin, Pathogenicity island: cag, cytotoxin associated gene A+genes related to bacterial secretion
- $Cag^{+}$ HP is much more associated with peptic ulcer disease than $Cag^{(--)}$ HP.

# Pathogenesis

- Motility – it moves into the mucus and produces adhesins on gastric epithelial cells (not intestinal epithelial cells)
- Urease production, breaks down the urea to ammonia which buffers the pH around the bacterium.
- Persists, escape defense mechanisms – SOD, catalase, Urease. Breack down free radicals



# Pathogenesis

- *H pylori* invade the epithelial cell surface to a certain degree
- Toxins and LPS may damage the mucosal cells
- $NH_3$ produced by the **urease** activity may also damage the cells

# Epidemiology

- Over 50% worlds population is infected.
- 70-80% in developing countries.
- Human genomic sequencing suggest that they got infected 58000 years back.
- More common in low socioeconomic status
- Humans are major reservoir
- Housing density, crowded conditions in the home, number of siblings, sharing a bed, and lack of hot running water

## Person to person transmission

- Gastro oral route
- Feco oral route
- Oral - Oral route

**Vomitus 100 fold higher than saliva and stool**

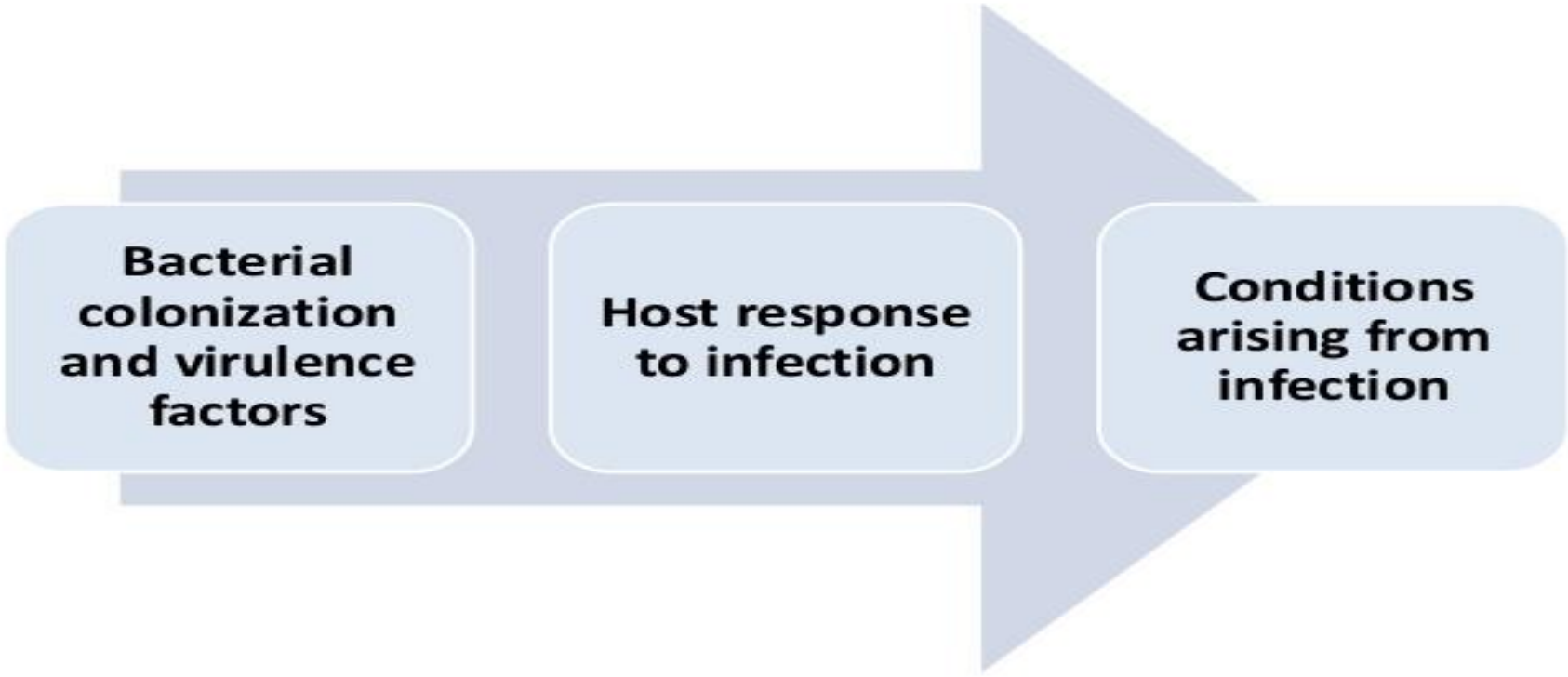
## Contaminated water

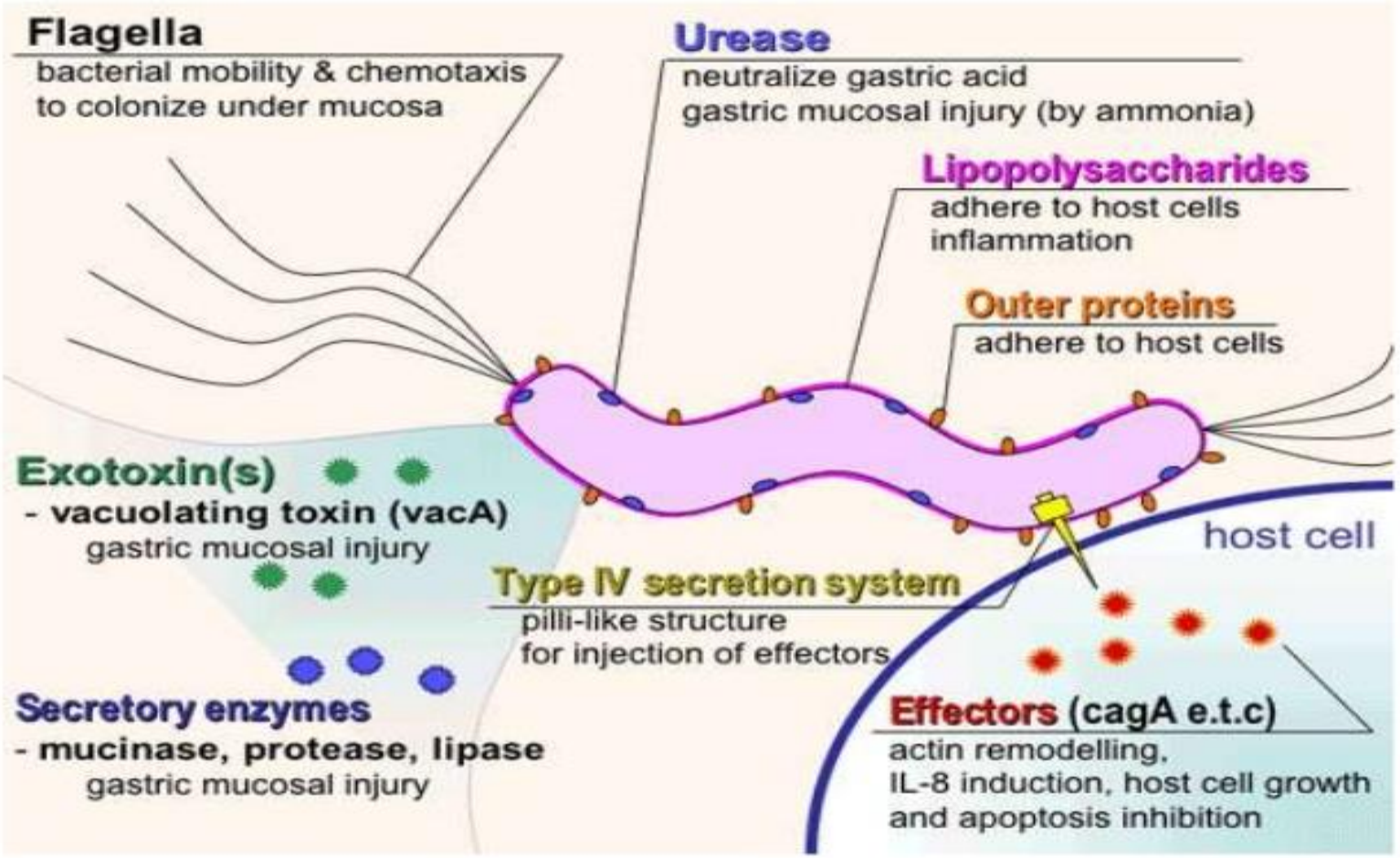
- developing countries

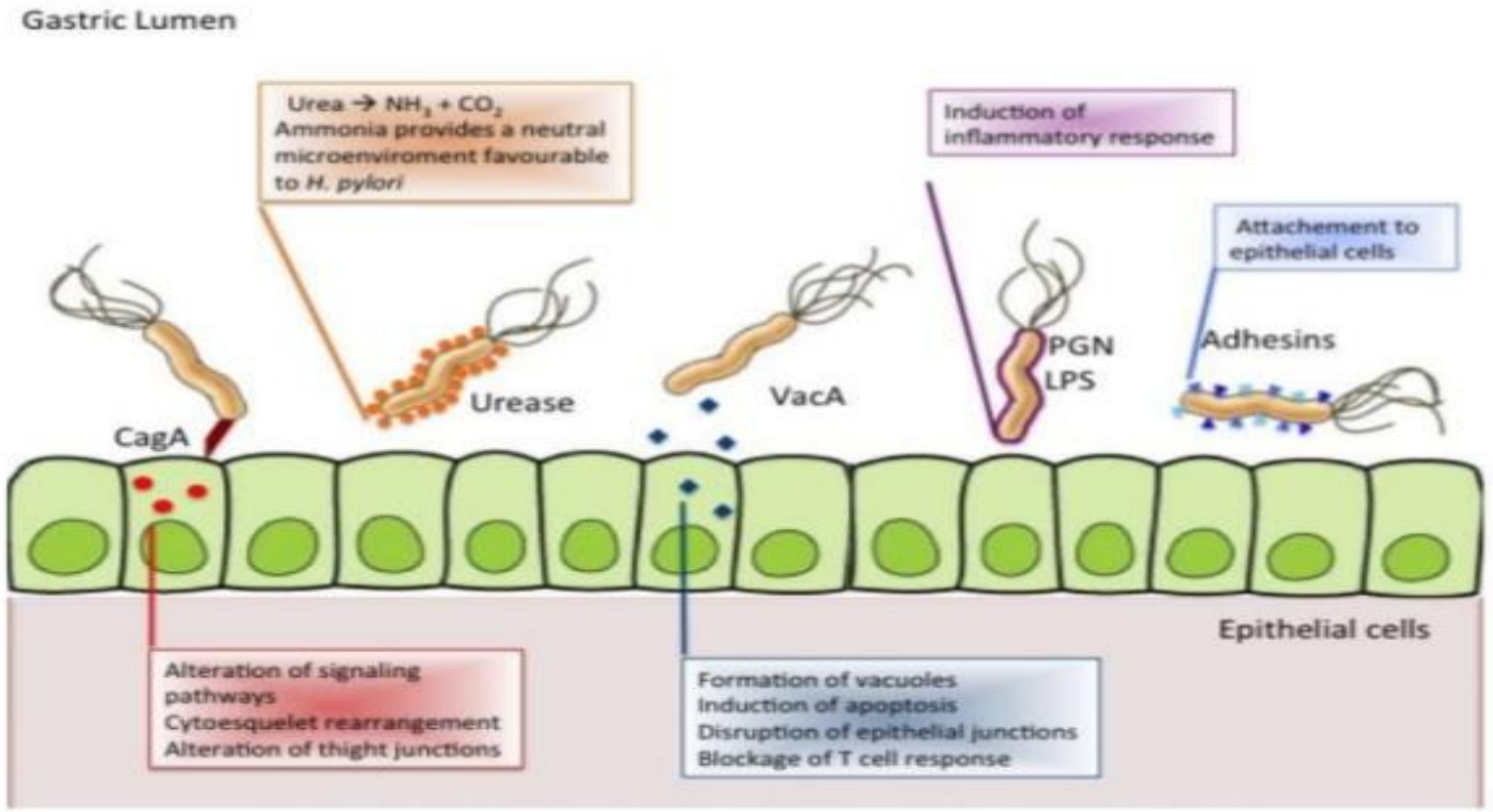
## Iatrogenic

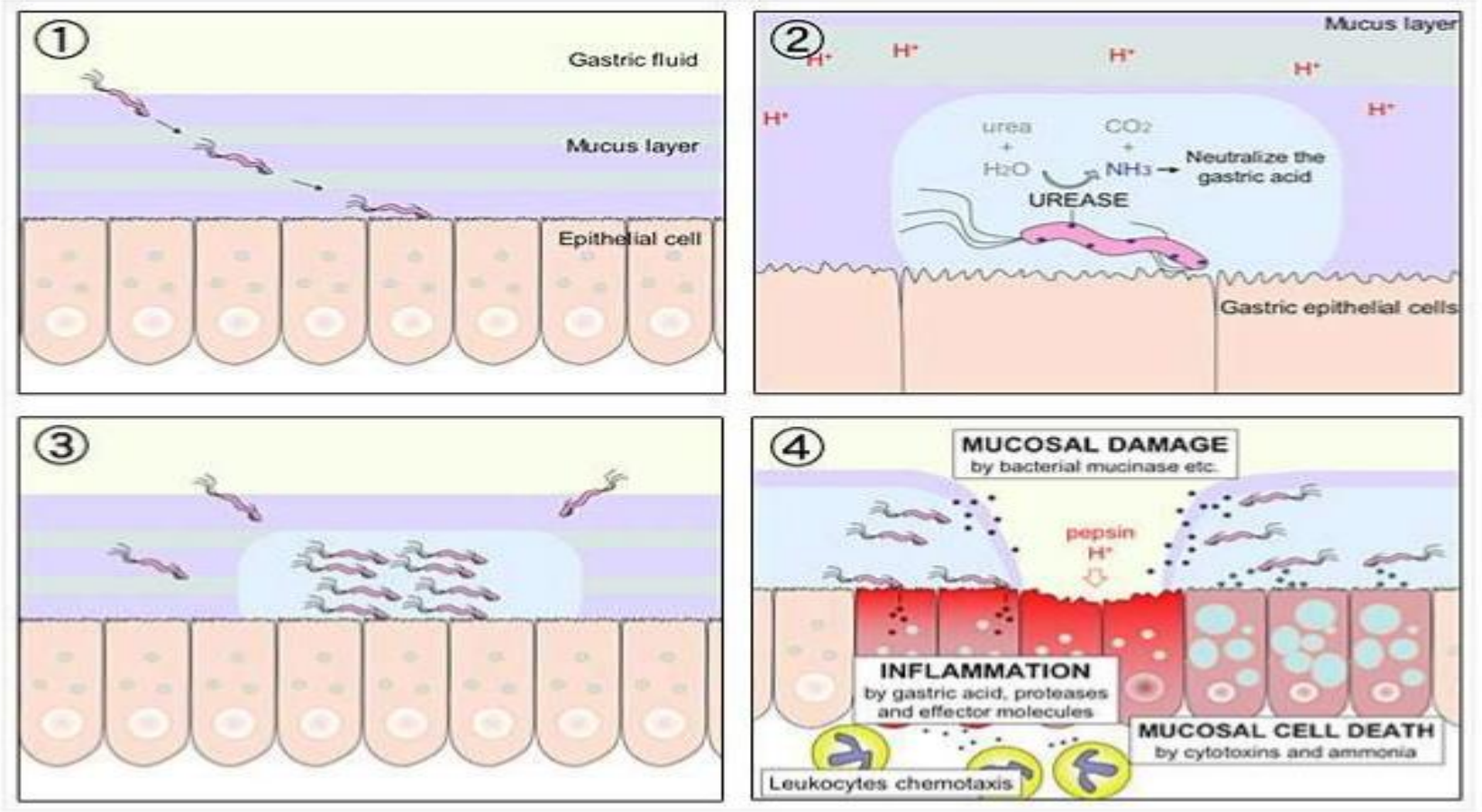
- inadequately disinfected endoscopic devices

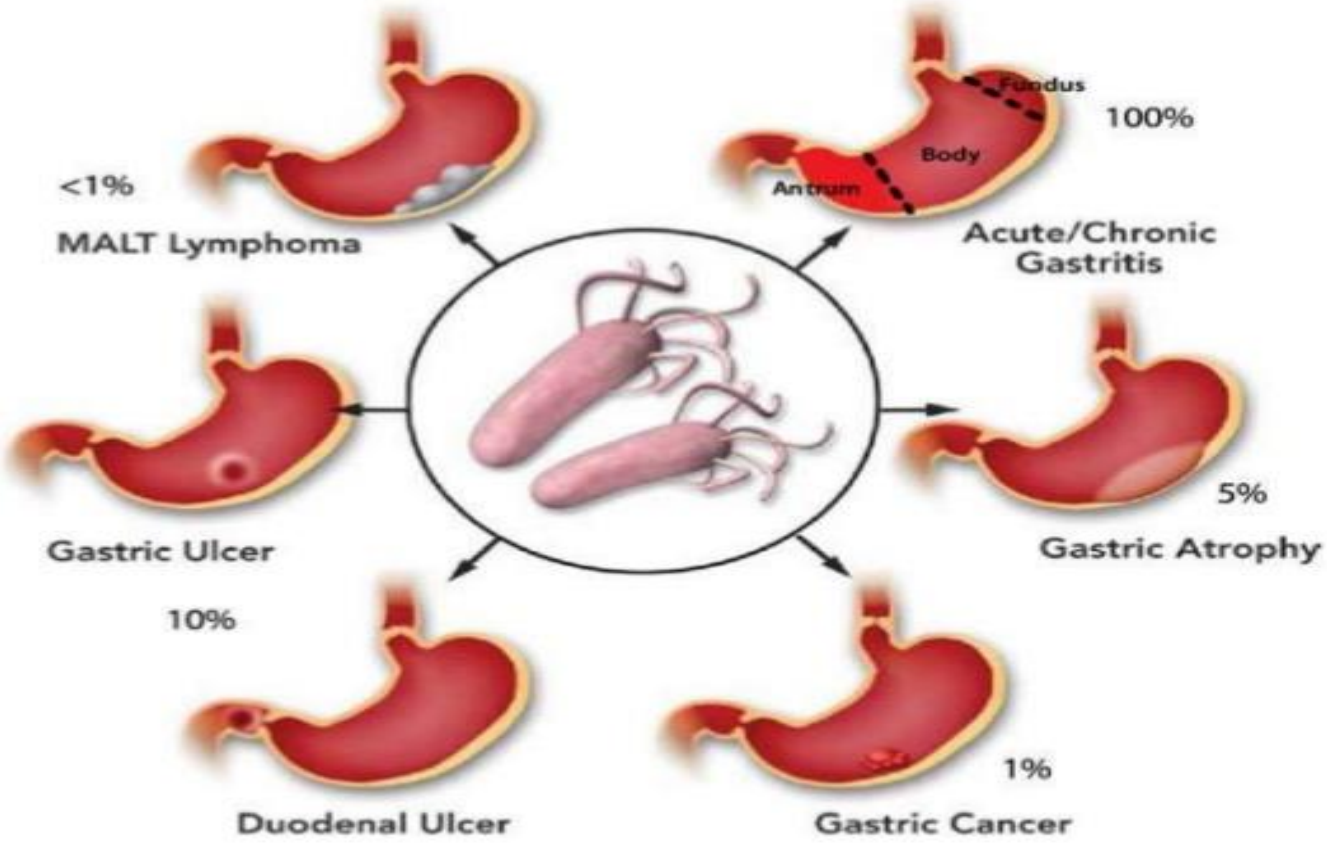
# Pathogenesis




Flagella
bacterial mobility & chemotaxis
to colonize under mucosa
Urease
neutralize gastric acid
gastric mucosal injury (by ammonia)
Lipopolysaccharides
adhere to host cells
inflammation
Outer proteins
adhere to host cells
Exotoxin(s)
- vacuolating toxin (vacA)
gastric mucosal injury
Type IV secretion system
pilli-like structure
for injection of effectors
host cell
Secretory enzymes
- mucinase, protease, lipase
gastric mucosal injury
Effectors (cagA e.t.c)
actin remodelling,
IL-8 induction, host cell growth
and apoptosis inhibition


Gastric Lumen
Urea → $NH_3$ + $CO_2$
Ammonia provides a neutral microenviroment favourable to *H. pylori*
Induction of inflammatory response
Attachement to epithelial cells
PGN
LPS
Adhesins
Urease
VacA
CagA
Epithelial cells
Alteration of signaling pathways
Cytoesquelet rearrangement
Alteration of thight junctions
Formation of vacuoles
Induction of apoptosis
Disruption of epithelial junctions
Blockage of T cell response


①
Gastric fluid
Mucus layer
Epithelial cell
②
Mucus layer
H+
urea
+
H2O
CO2
+
NH3
Neutralize the gastric acid
UREASE
Gastric epithelial cells
③
④
MUCOSAL DAMAGE
by bacterial mucinase etc.
pepsin
H+
INFLAMMATION
by gastric acid, proteases and effector molecules
MUCOSAL CELL DEATH
by cytotoxins and ammonia
Leukocytes chemotaxis


<1%
MALT Lymphoma
Fundus
Body
Antrum
100%
Acute/Chronic
Gastritis
Gastric Ulcer
10%
5%
Gastric Atrophy
Duodenal Ulcer
1%
Gastric Cancer

# Tests for Hp Infection

## Non Endoscopic Tests

- Serology (qualitative or quantitative Ig G)
- Urea breath test
- Stool antigen test

## Endoscopic Tests

- Histology
- RUT
- Culture
- PCR assay