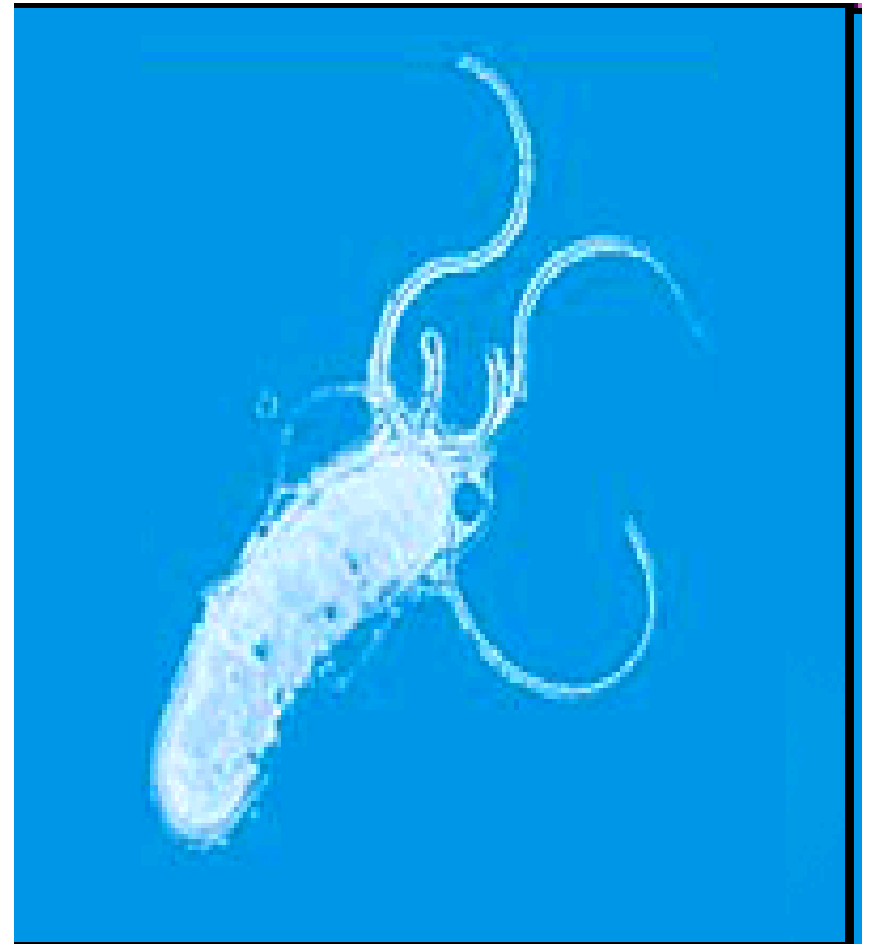


**Lecture topic:**  
**HELICOBACTER, CAMPYLOBACTER AND MISCELLANEOUS  
BACTERIA**

# Helicobacter pylori

- Helicobacter pylori is a spiral gram negative bacteria.
- It has a multiple polar flagella above the pole and motile



# Culturing H.pylori

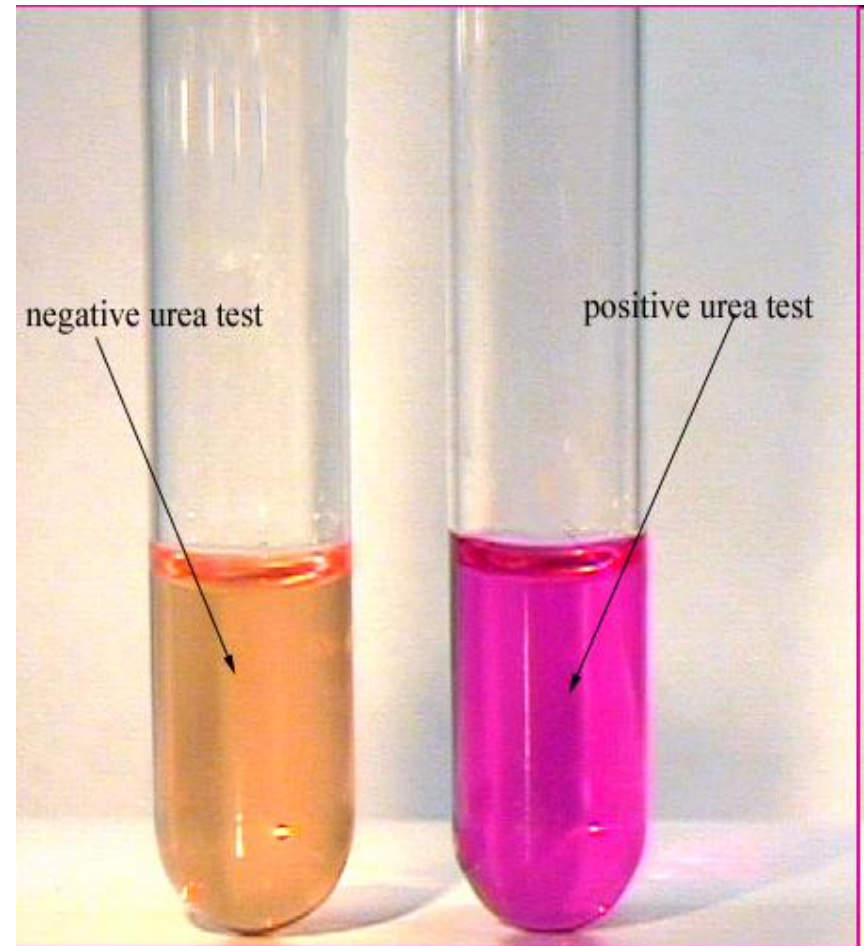
- H.pylori grows on Skirrow's medium with
  - 1 Vancomycin,
  - 2 Polymyxin
  - 3 TrimethoprimGrows in 3 -6 days at 37<sup>0</sup>c  
Colonies appear  
Translucent 1-2 mm in diameter  
Optimal growth occurs in  
Microaerophic environment



# Biochemical Characters

- Motile
- Catalase +
- Oxidase +

Strong producer of  
**Urease**



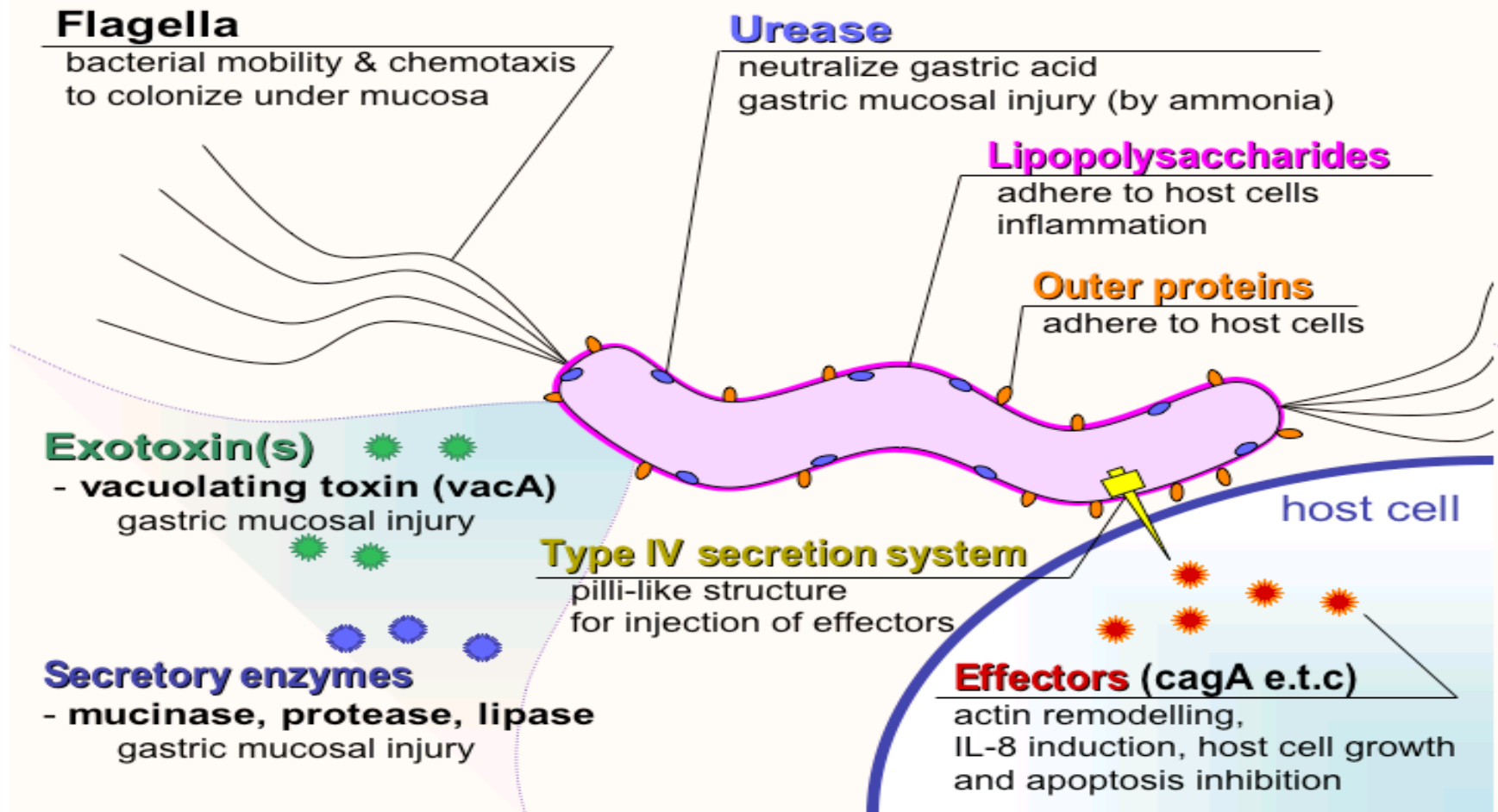
# Pathology and Pathogenesis

- H.pylori is found in the deep mucus layer
- Grows optimally at pH 6.0 to 7.0
- But gastric mucosa has a strong buffering in spite of lower pH on the lumen side of stomach
- H.pylori also produces a protease that modifies the gastric mucus and further reduces the ability of acid through the mucus

# Virulence factors

- **vacA (vacuolation associated) cytotoxin, Pathogenicity island: cag, cytotoxin associated gene A+genes related to bacterial secretion**
- **Cag<sup>+</sup> HP is much more associated with peptic ulcer disease than Cag<sup>(--)</sup> HP.**

# Mechanisms in Pathogenicity



# Factors contributing to Peptic ulceration

- There is a strong association between presence of H.pylori infection and peptic ulceration
- Mucosal inflammation and damage involves both bacterial and host factors



# *Clinical Manifestations*

- Acute infection

Upper Gastrointestinal illness

Nausea

Pain

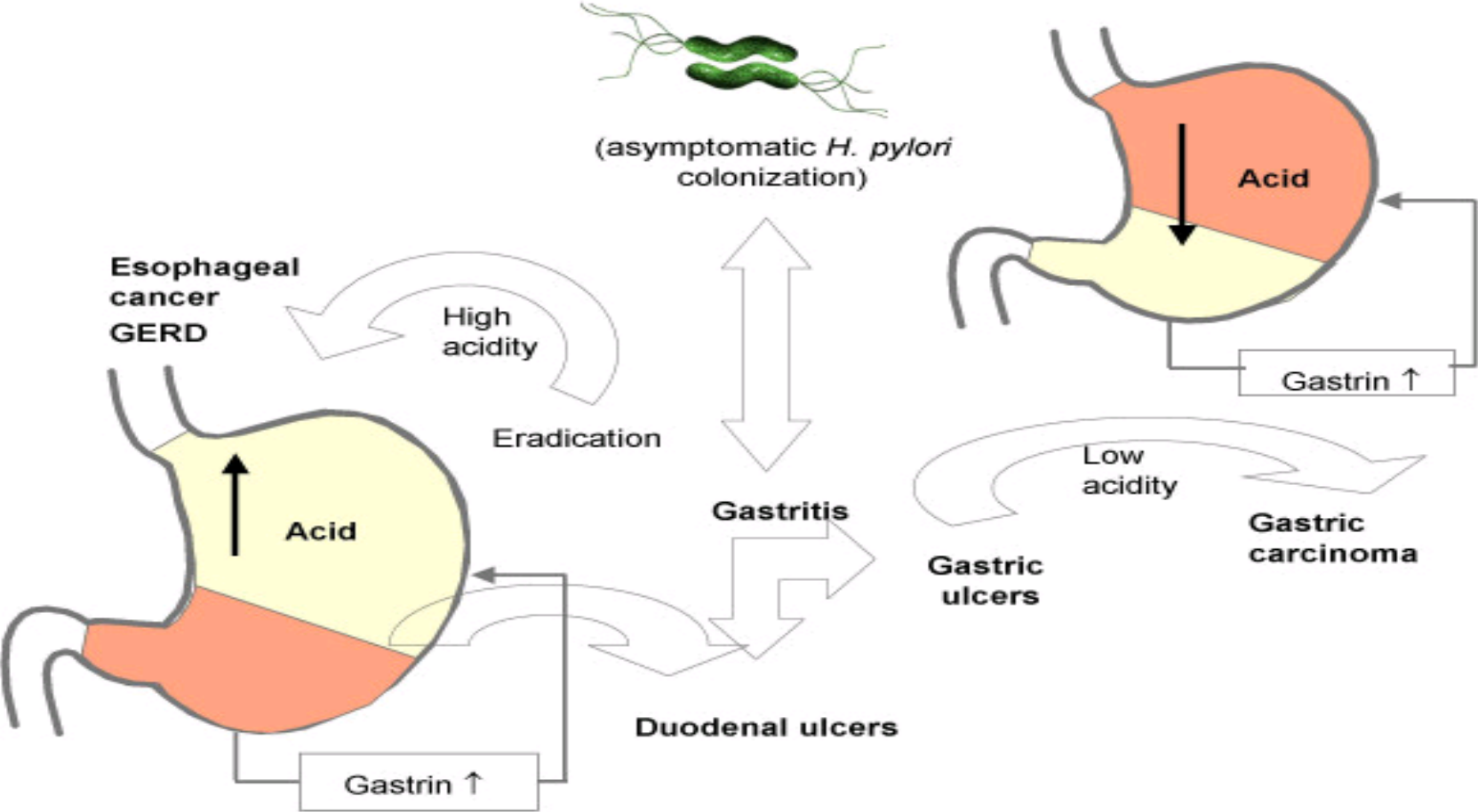
Fever – very occasionally

Acute symptoms lasts for < 1 week,

May extend upto 2 weeks

**Infection last for years, decades or even lifetime**

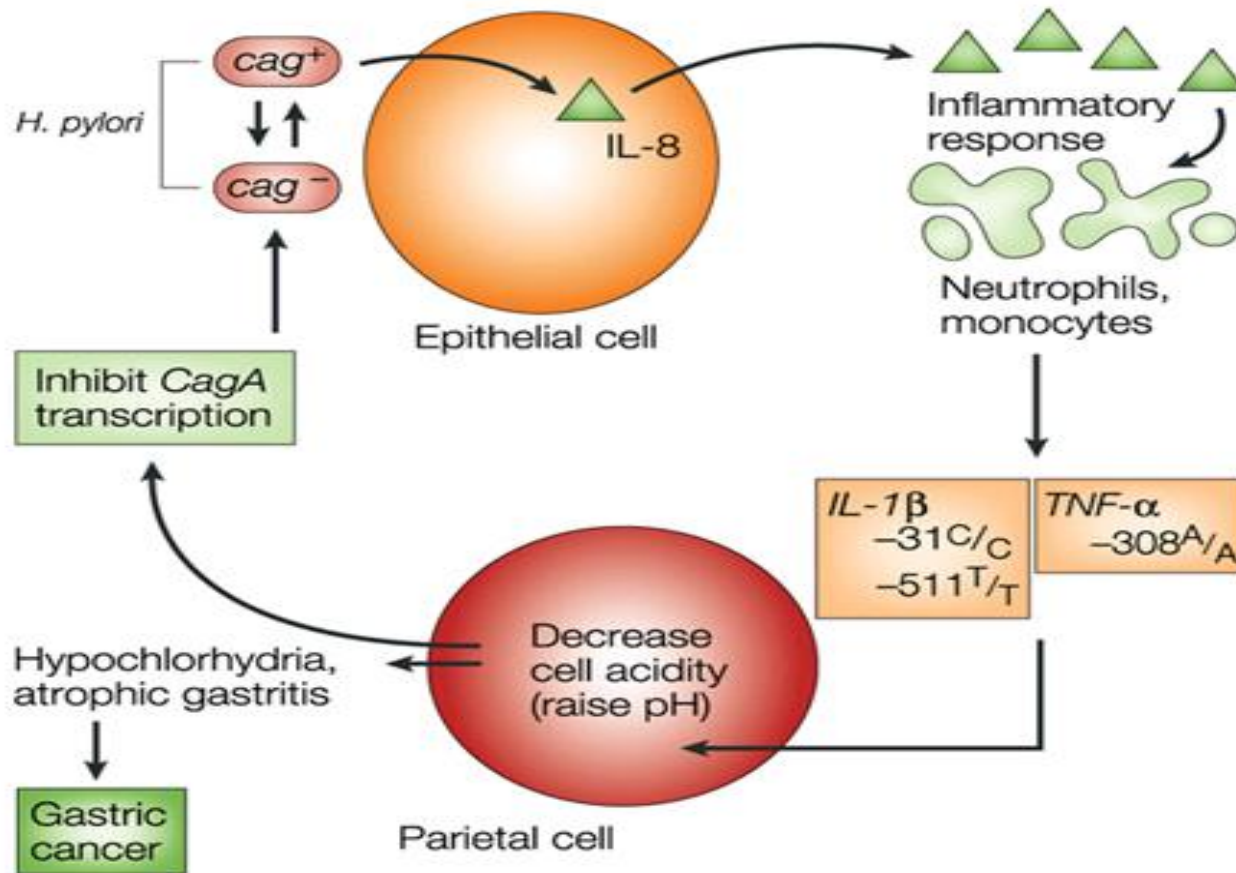
# Consequences of *H. pylori* Infection



# Association of Duodenal and Gastric ulcers in H.pylori

- About 90 % of patients with Duodenal ulcer, and 50- 80 % of gastric ulcers are associated with H.pylori infection.
- H.pylori may have greater role in Gastric carcinoma and Lymphomas

# Mechanism of Cancer in H.pylori



# Laboratory Diagnosis

- **Specimens for histopathology** – Gastric biopsy specimens can be used for Histological examination
- Specimens obtained after Gastroscope, Biopsy, routine stains will demonstrate Gastritis and special stains show curved spiral organisms
- Specimens collected in sterile saline mixed are used for culturing

# Culturing for H.pylori

- Culturing of H.pylori needs specific conditions
- Media
  - Skirrow's Medium
    - with
      - Vancomycin
      - Polymyxin B
      - Trimethoprim
  - Chocolate Medium with
    - Vancomycin, Nalidixic acid
    - Amphotericin



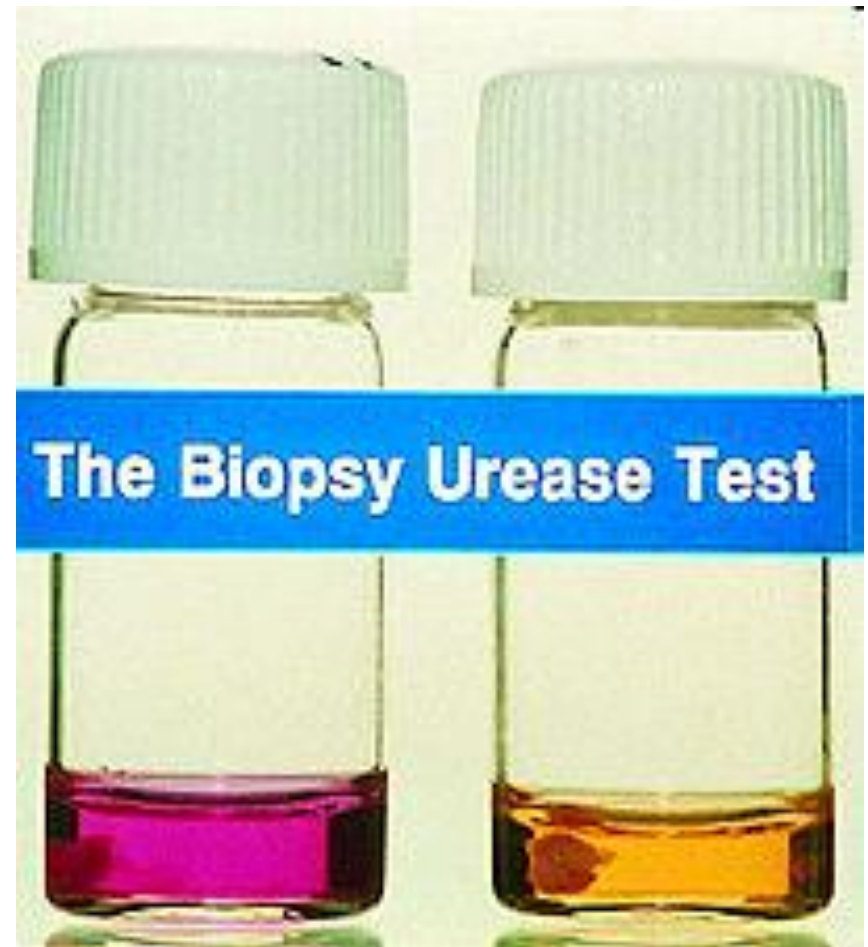
# Serology

- The detection of Antibodies in active infection is useful
- But the tests are limited utility as antibodies persist even after H.pylori infection is eradicated.
- Several commercial kits are available, but lacks the role in identifying acute infections.



# Special Tests for H.pylori

- Rapid tests for detection of Urease activity are widely used in presumptive identification of Gastric Biopsy specimens.
- Gastric Biopsy can be placed into urea containing medium with color indicator.
- **If H.pylori is present the Urease rapidly splits urea and resulting shift in pH yields a color change in the medium**





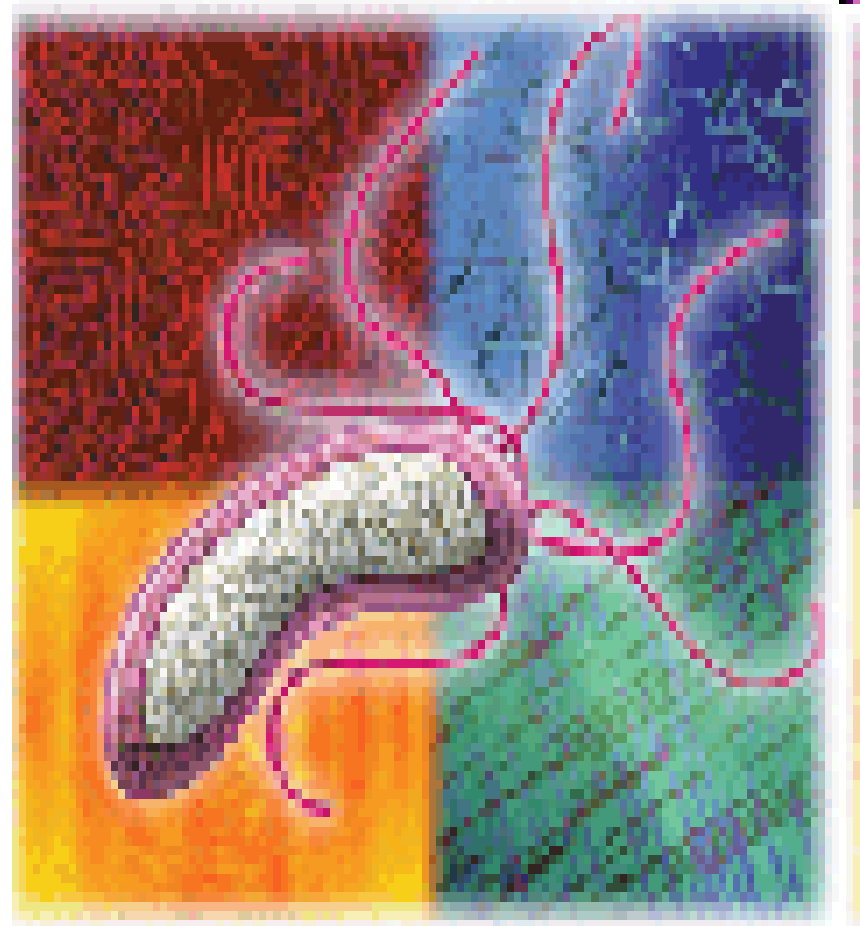
# Urea Breath Test

- *H. pylori* infection can be detected in the exhaled breath using this special test. This test is positive only if the person has a current infection. Sensitivity and specificity of this test ranges from 94-98%.
- In this test  $^{13}\text{C}$  or  $^{14}\text{C}$  labelled urea is ingested by patients
- **If *H.pylori* is present the urease activity generates labelled  $\text{CO}_2$  that can be detected in the patients exhaled breath**



# Antigen Detection Test in Stool

- Detection of H.pylori antigen in stool is appropriate test in patients with H.pylori infection
- **Absence of antigen indicates cure of Infection after Chemotherapy.**



# Treatment

- Triple therapy has prompt response, contain a combination of following drugs
  - 1 Metronidazole
  - 2 Bismuth subsalicylate or Bismuth sub citrate
  - 3 Amoxicillin or Tetracyclesadministered upto 14 days  
Eradicates H.pylori  
In 70 – 95 % of patients  
Acid suppressing agent is supporting

# Other Drug Combination

- **Other alternatives**

Proton pump inhibitor directly inhibit

H.pylori

Combined with

Amoxicillin

Clarithromycin or Amoxicillin

And Metronidazole

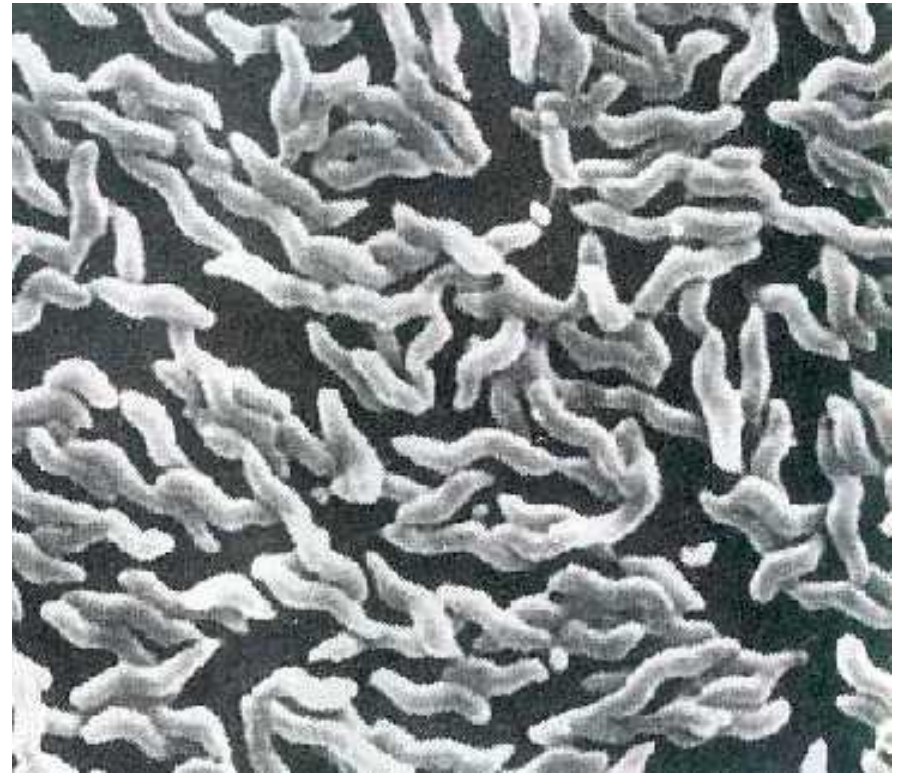
***CAMPYLOBACTER***

# *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.
- Motile, with a single polar flagellum
- No spore & no capsule



# Culture

- An atmosphere with reduced O<sub>2</sub> (5% O<sub>2</sub>) with added CO<sub>2</sub> (10% CO<sub>2</sub>)
- At 42 °C (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  $10^4$  organisms)
- Multiply in the small intestine → invade the epithium → produce inflammation → cause bloody stools →
- Occasionally, the bloodstream is invaded →

# ***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**
- occasionally**
- **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)

# Control

- The source of infection may be food (eg, milk, under-cooked fowl) or contact with infected animals or humans and their excreta.

MISCELLANEOUS BACTERIA:

LISTERIA

LEGIONELLA

RAT BITE FEVER

*Legionella pneumophila*

# CLINICAL FEATURES

- Legionellosis
- 2 distinct patterns of illness
  - Legionnaires disease
  - Pontiac fever

## 1. Legionnaires Disease

- Epidemic/ sporadic
- Fever, non productive cough, dyspnoea
- Rapidly progressing to pneumonia
- Diarrhoea and encephalopathy are common
- Immunocompromised
- Case fatality 15 – 20 %



## 2. Pontiac fever

- Milder
- Non fatal
- Influenza like illness – fever chills, myalgia, headache
- Outbreaks may occur
- Affects previously healthy people.

# MORPHOLOGY

- Gram negative Coccobacilli.
- Motile – Polar or subpolar flagella.
- better by Silver impregnation  
DFA  
Immunoelectronmicroscopy
- Non capsulated, non sporing, non acid fast.

# CULTURE

- Fastidious
- Requires cysteine and iron for primary isolation
- **BCYE** – buffered charcoal yeast extract with L-cysteine
- BCYE with vancomycin, polymyxin, anisomycin
- MHA with 1% Isovitalex
- Incubation in 5% CO<sub>2</sub> and high humidity
- Slow growth – 3- 6 days to appear
- Circular, grey, low convex, 1-2 mm in diameter

# Epidemiology

- Widely distributed in natural waters – stagnant waters, hot springs
- Survive and multiply inside free living amoebae
- Also multiply in artificial aquatic environment
- Can also survive in soil
- **Transmission**
  - Inhalation of Aerosolised Legionellae by cooling towers, ACs, spa baths, shower, shower heads, industrial coolants, respiratory ventilators
  - No animal reservoir
  - No carrier state
  - No man to man transmission

- **Outcome of Infection:**

- Infecting dose

- Virulence of Strain

- Host factors – Smoking

- Alcohol

- Advanced age

- Intercurrent illness

- Hospitalization

- Immunodeficiency

- Intracellular (CMI is protective).
- Antigenic structure
  - flagellar and somatic antigens
  - 14 serogroups (type 1)
- Virulence determinants
  - Endotoxin
  - Hemolysin
  - Cytotoxin
  - protease

# LAB DIAGNOSIS

- SPECIMENS
  - Sputum, bronchial aspirate, pleural fluid, lung biopsy
- Microscopy
  - Fluorescent antibody technique using monoclonal or polyclonal sera
- Culture
  - Identified by colonial morphology .

- Detection of Legionella Antigen
  - ELISA
  - Rapid and specific test
- Detection of Legionella Antibodies
  - Antibodies develop 6-8 days after the onset of clinical illness
  - Detected by indirect fluorescent antibody test with heat or formalin fixed antigens
  - > 256 titre is significant
- PCR



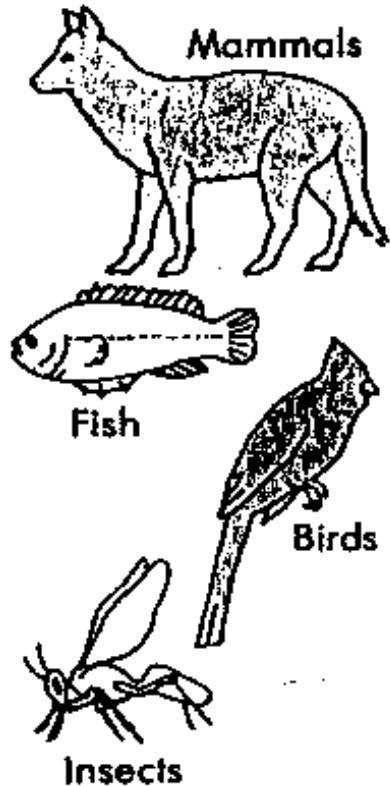
# TREATMENT

- Erythromycin and Rifampicin
- Generally required in Legionnaires disease
- Pontiac fever is self limiting
- No vaccine available.

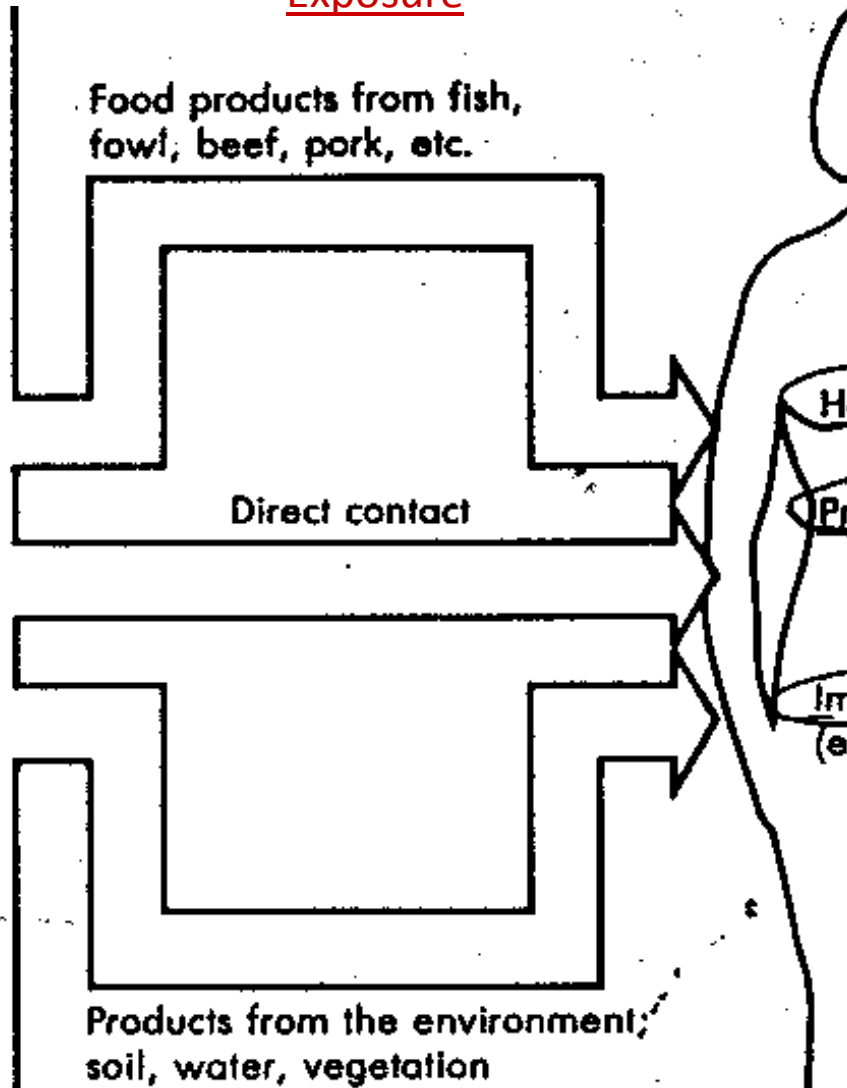
# LISTERIA

# Epidemiology of Listeria Infections

## Natural Reservoirs



## Common Routes for Human Exposure



## Population at Greatest Risk

Healthy children and adults  
Asymptomatic carriage

Pregnant women  
Asymptomatic carriage  
Septicemia  
Neonatal disease

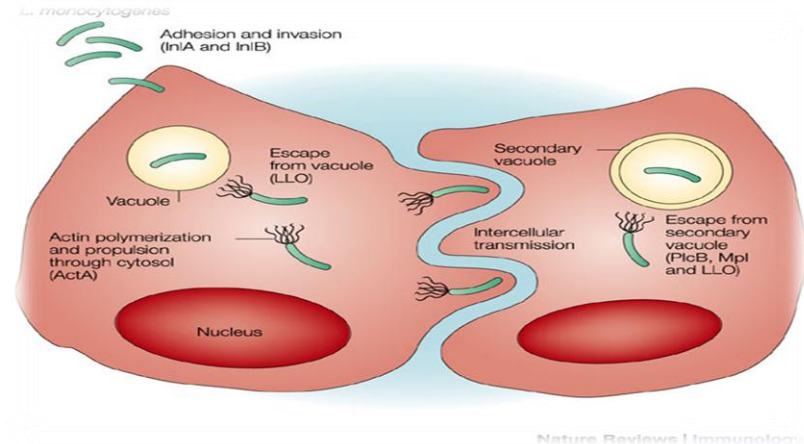
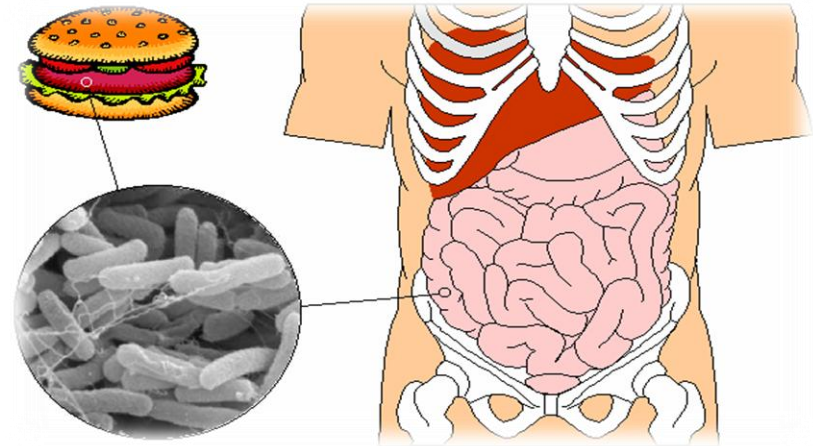
Immunocompromised  
(e.g., cancer or transplant patients)  
Asymptomatic carriage  
Meningitis  
Septicemia  
Other infections

# EPIDEMIOLOGY

- Widely distributed in nature.
- Most human infections are caused by serovar 1/2a or 1/2b and 4b.
- Outbreaks of foodborne listeriosis have been known as the bacteria can overcome the food preservation process due to its ability to grow at refrigeration temperature, low Ph and high salt concentration.

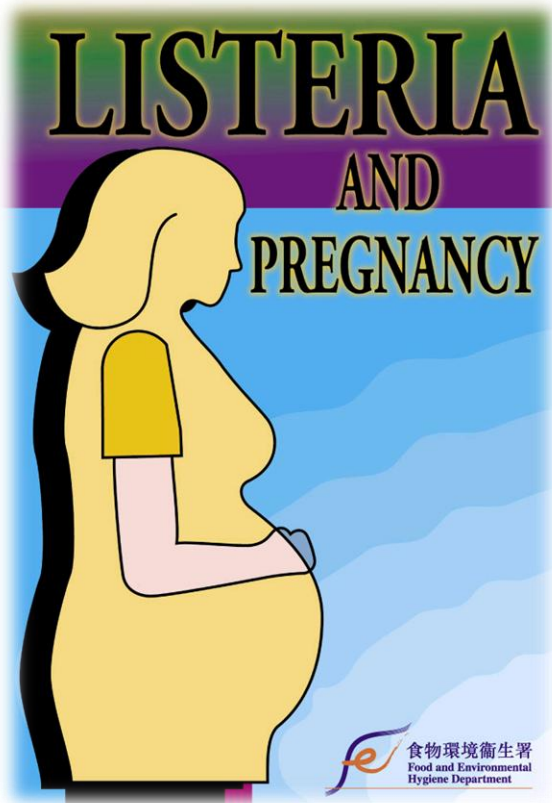
# Pathogenesis and Pathology

- enters through the GI tract -cheese or vegetables.
- The cell wall surface protein called Interanalin interacts with E – CADHERIN and enters into epithelial cells.
- Bacteria produce Listeriolysin .
- CMI(**Intracellular pathogen**).



# Pathogenecity

- Infection of **pregnant women** may lead to abortion or stillbirth if intrauterine transmission occurs.
- It may cause **meningitis or sepsis** in neonates due to perinatal transmission, which can be of early or late onset.
- In the **immunocompromised and elderly**, it can cause meningoencephalitis



# Symptoms

- Fever
- Muscle ache
- GI Sx: Nausea, diarrhea
- Pregnant women: mild flu-like Sx, **miscarriage, still birth, premature delivery**, or infected newborn.
- Lethargy
- irritability
- **Meningitis.**  
(serotype **IV b** , significant mortality rate)
- Pneumonia
- Sepsis.



# Culture and Growth Characteristics

- Grows on Muller Hinton agar with sheep blood as enrichment.
- Small zone of Hemolysis can be observed around and the underneath of the colony.
- Specimens are enriched if the tissues are kept at 4<sup>0</sup>c and plated on the media  
( **Cold enrichment** )



ASM MicrobeLibrary.org © Buxton

- *Listeria monocytogenes* is a short, non-sporing, Gram positive bacillus.
- exhibits tumbling motility when grown at 25° C
- Non motile at 37° C.
- It is aerobic or microaerophilic.
- catalase positive
- Ferments glucose, maltose, L-rhamnose and alpha methyl D-mannoside, producing acid without gas.
- Experimental inoculation in rabbits causes marked monocytosis( hence the name monocytogenes).
- Instillation into the eyes of rabbits produces keratoconjunctivitis ( Anton test )

# Antibiotic Treatment

- Ampicillin
- Erythromycin
- Trimethoprim – Sulphamethoxazole
- Cephalosporins and Fluroquinoles are not active against *l.monocytogens*.

# **RAT BITE FEVER**

- Caused by Rat bite/Consumption of raw milk or water contaminated by rats
- Relapsing fever, Rash, Arthralgia
- *Streptobacillus moniliformis* / *Spirillum minus*
- Both are natural parasites of rodents

## *Streptobacillus moniliformis*

**Morphology:** Highly pleomorphic, **Gram negative, non motile**, non capsulate bacillus

String of beads appearance, Readily develops into L forms

**Culture:** Fastidious - requires blood, serum, ascitic or other body fluids.

-5- 10 % CO<sub>2</sub> and moist atmosphere

**Biochemicals:**

Cat - , Oxd - , Nitrate -, Urease -, Indole - Glucose – acid.

**Serology:** Agglutination/CFT

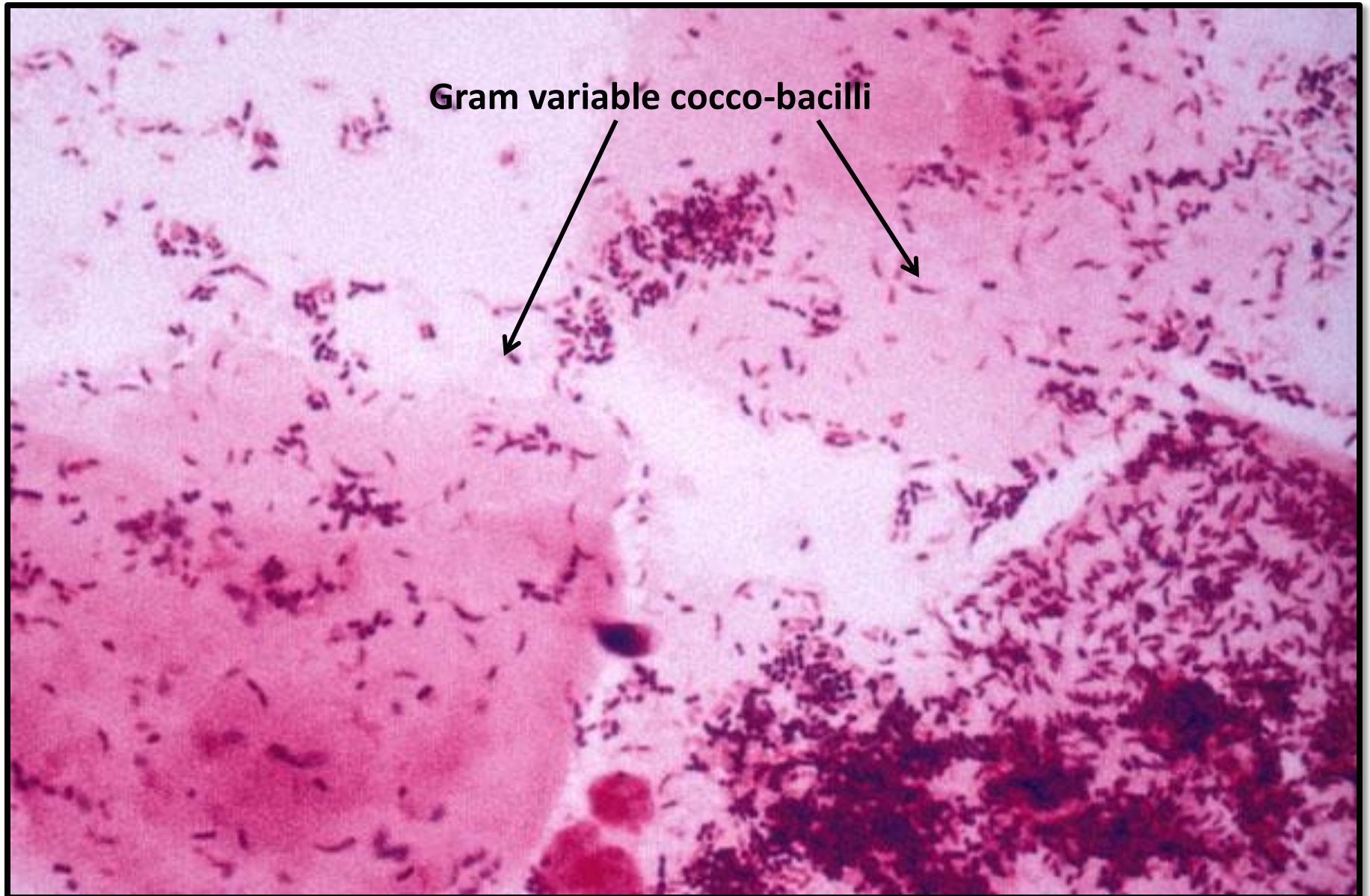
**Animal inoculation** – Mice are highly susceptible to infection

I/P in mice – fatal progressive condition with swelling of feet and legs

## *Spirillum minus*

- **Morphology:**
  - spiral
  - Gram negative, but better visualised by
    - Giemsa/ Fontana stain
    - Dark field microscopy
  - actively motile bacillus (1-7 flagella at each pole)
- **Culture:**
- Not been cultivated in lab. Media
- i/p inoculation into mice, guinea pig.
- **Treatment:** Penicillins and tetracyclins

# Bacterial Vaginosis





<b>Grade of flora</b>	<b>Definition of flora</b>	<b>Description (By Ison and Hay)</b>
0	Normal(found during or immediately after post-antibiotic therapy)	Epithelial cells only; no bacteria seen
I	Normal	Lactobacillus morphotype only
II	Intermediate	Reduced lactobacillus morphotype with mixed bacterial flora
III	Bacterial vaginosis	Mixed bacterial flora with few or absent lactobacillus morphotype
IV	Normal in asymptomatic women	Epithelial cells covered with gram-positive cocci with few or absent lactobacillus morphotype

# Amsel's Criteria

- Demonstration of **clue cells** on a saline smear is the most specific criterion for diagnosing BV.
- A pH greater than 4.5 indicates infection, and pH may be elevated in up to 90% of patients with BV. (**loss of acidity**)
- Characteristic **discharge** appearance is thin, gray, and homogeneous.
- The **whiff test** may be positive in up to 70% of BV patients.

## Nugent Scoring System (0-10) for Gram-Stained Vaginal Smears

Score	<i>Lactobacillus</i> morphotypes		<i>Gardnerella</i> and <i>Bacteroides</i> morphotypes		Curved gram-variable rods
0	4+		0		0
1	3+		1+		1+ or 2+
2	2+	+	2+	+	3+ or 4+
3	1+		3+		-
4	0		4+		-

**Scoring Based on Morphotypes per High Power Field:** 0 = 0; 1+ = <1; 2+ = 1-4; 3+ = 5-30; 4+ = ≥30  
**Total Score:** 0-3 Normal; 4-6 Intermediate; 7-10 Bacterial Vaginosis

**Thank you**