

GRAM-NEGATIVE BACILLI

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GRAM-NEGATIVE BACILLI

It is divided into 3 clinical subgroups:

1. Act in the gut
2. Act in the respiratory tract
3. Those transmitted to humans by animal sources

Gram-negative bacilli associated with the intestinal tract

1. Pathogens that cause disease both inside and outside the digestive tract
2. Pathogens that cause disease only within the digestive tract
3. Pathogens that cause disease outside the digestive tract

Gram-negative bacilli of the digestive tract

- This group of bacteria is in the Enterobacteriaceae family.
- Many members of the Enterobacteriaceae are found in the large intestine of humans and other animals and constitute a large part of the normal flora in this region.

Enterobacteriaceae

- *Escherichia*
- *Shigella*
- *Salmonella*
- *Edwardsiella*
- *Citrobacter*
- *Yersinia*
- *Klebsiella*
- *Enterobacter*
- *Serratia*
- *Proteus*
- *Morganella*
- *Providencia*

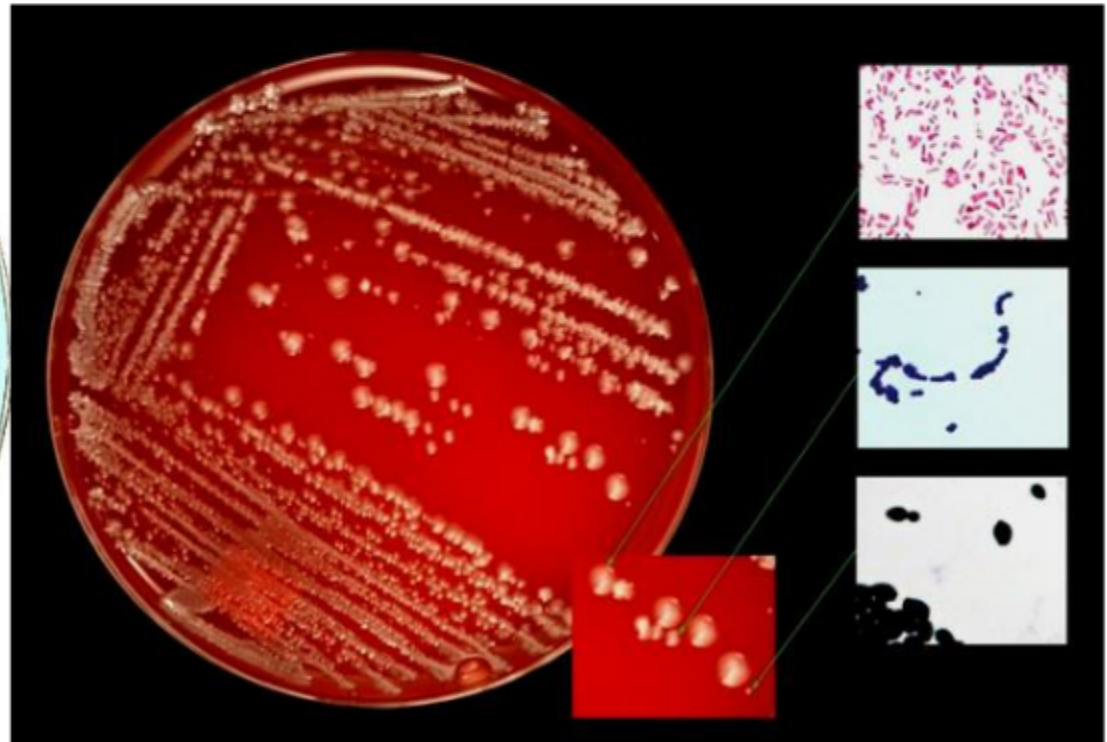


Bölüm	Enfeksiyon Kaynağı veya Yeri	Cins
18	Sindirim kanalı	
	1. Hem içi hem dışında	<i>Escherichia coli</i> , <i>Salmonella</i>
	2. Esas olarak içinde	<i>Shigella</i> , <i>Vibrio</i> , <i>Campylobacter</i> , <i>Helicobacter</i>
	3. Sadece dışında	<i>Klebsiella-Enterobacter-Serratia</i> grubu, <i>Proteus-Providencia</i> , <i>Morganella</i> grubu, <i>Pseudomonas</i> , <i>Bacteroides</i>
19	Solunum kanalı	<i>Haemophilus</i> , <i>Legionella</i> , <i>Bordetella</i>
20	Hayvansal kaynaklar	<i>Brucella</i> , <i>Francisella</i> , <i>Pasteurella</i> , <i>Yersinia</i>

Microorganisms belonging to ***Enterobacteriaceae*** are the most frequently isolated bacterial group from clinical specimens.

These bacteria;

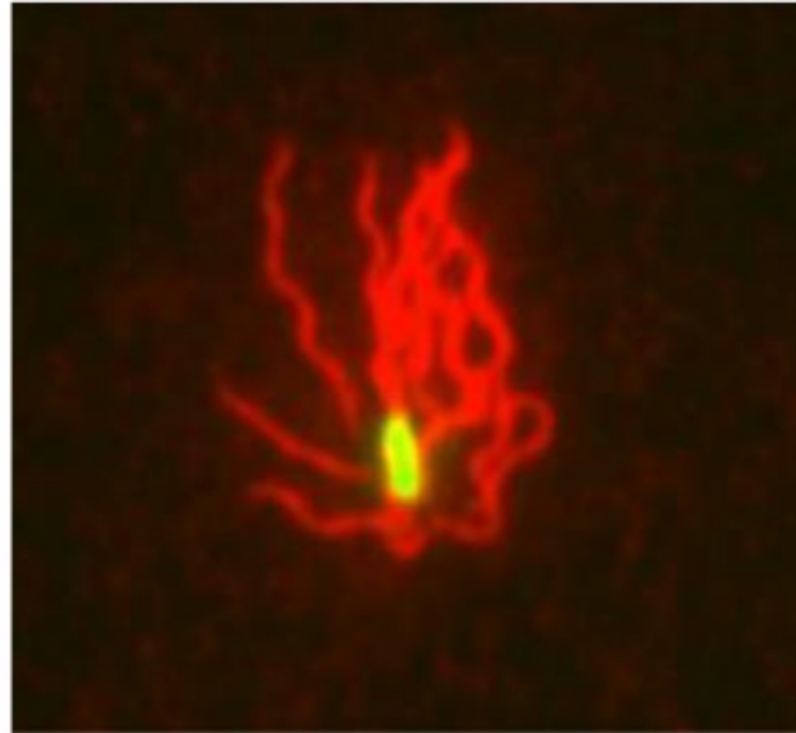
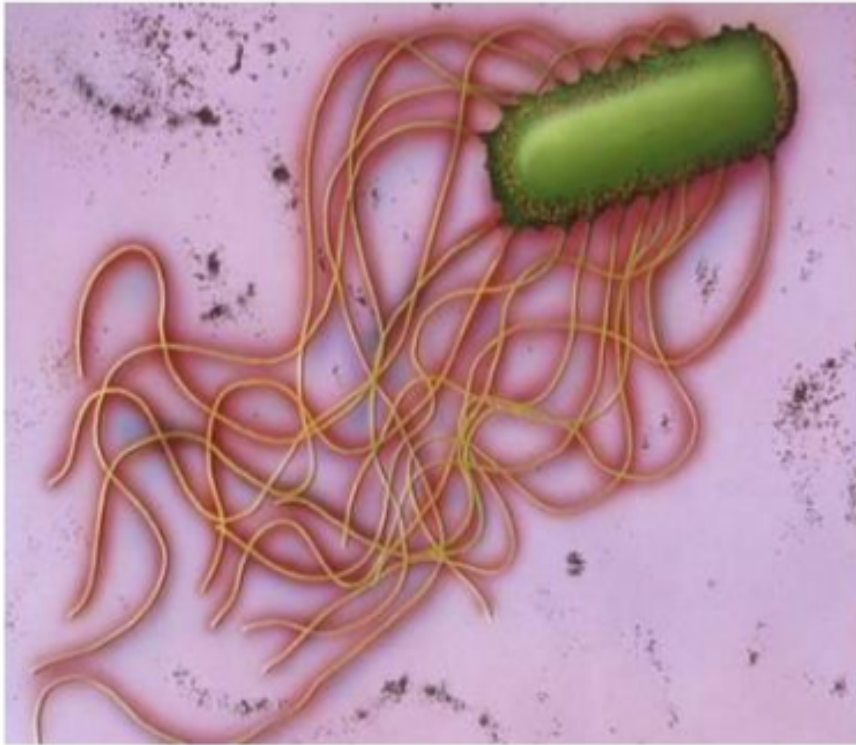
It is responsible for 70% of all urinary tract infections and 30-35% of all sepsis.



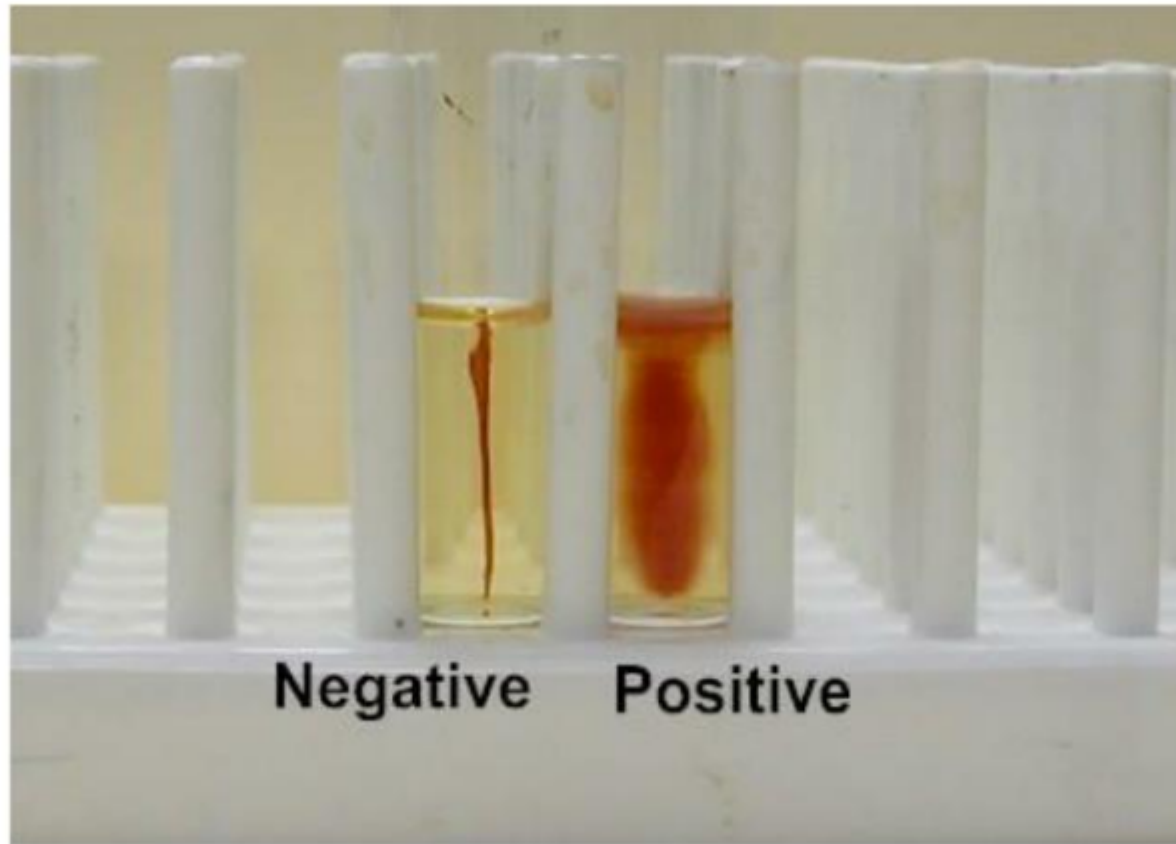
Features common to the Enterobacteriaceae family:

1. All are facultative anaerobes.
 2. All ferment glucose.
 3. None of them contain cytochrome oxidase.
 4. It reduces nitrates to nitrites as part of energy production processes.
- ✓ These features distinguish Enterobacteriaceae from non-fermenting organisms.

They are motile with their flagels. Only *Klebsiella* and *Shigella* are dormant.



Movement



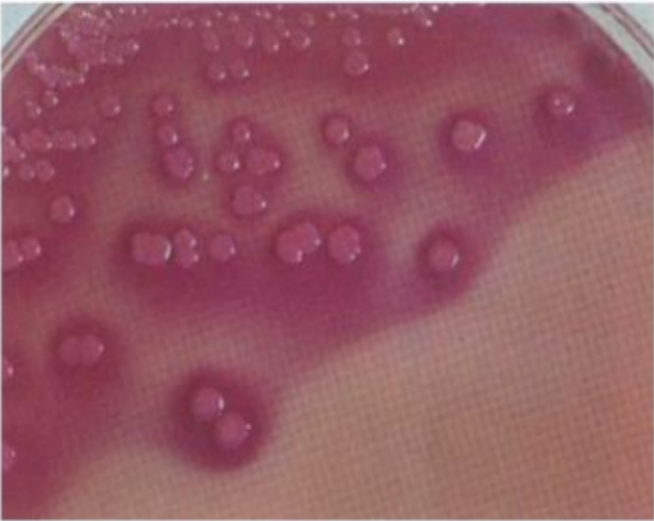
ORGANISM	DISEASES
Lactose Positive	
<i>Escherichia coli</i>	Diarrhea, sepsis, urinary tract infection, neonatal meningitis
<i>Klebsiella, Serratia, Citrobacter, Enterobacter</i> species	Opportunistic infections (e.g., pneumonia, sepsis, neonatal meningitis)
Lactose Negative	
<i>Salmonella</i> species	Diarrhea, typhoid fever, bacteremia; localized infections in bone, meninges, liver
<i>Shigella</i> species	Dysentery
<i>Proteus</i> species	Urinary tract infection
<i>Yersinia</i> species	Plague, diarrhea, mesenteric lymphadenitis

Laboratory Diagnosis

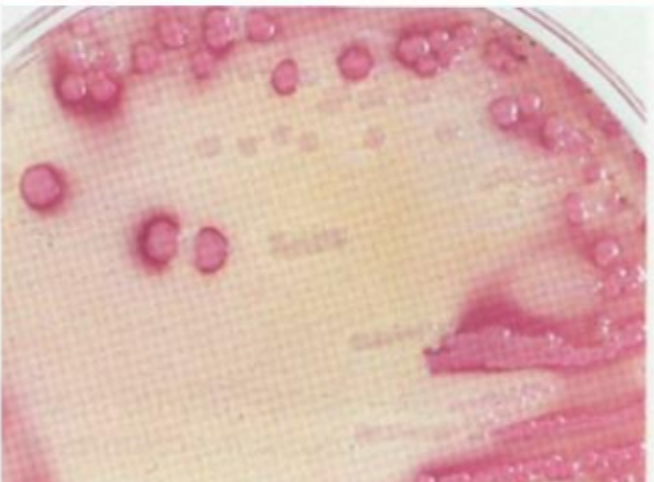
1. Blood agar
2. MacConkey agar and
3. EMB agar (Eosin-methylene blue)
4. Three sugar-iron agar (TSI)

Media Used and Colony Characteristics

MacConkey Agar

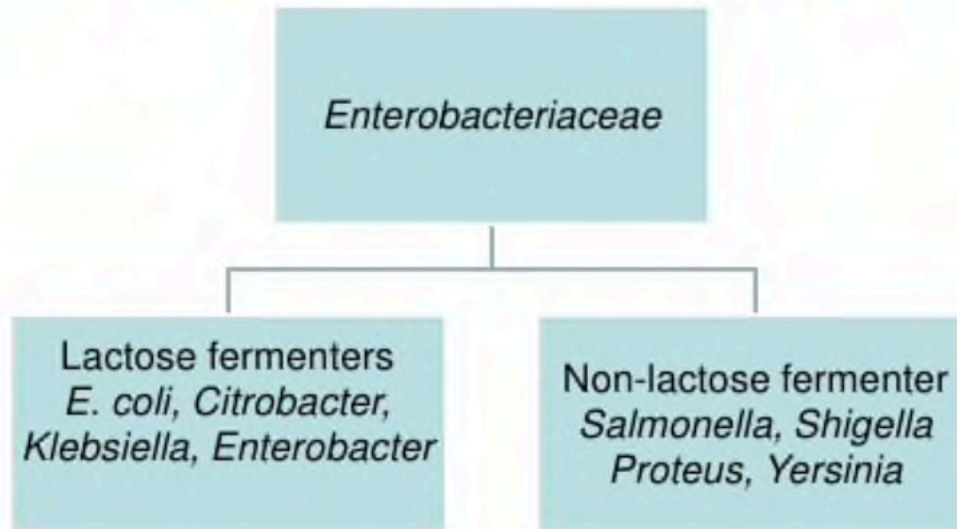


It is a selective-distinctive medium. The only carbohydrate is lactose. Crystal violet and bile salts inhibit the growth of gr(+) bacteria. The indicator is neutral red.



Escherichia, Klebsiella, Enterobacter MacConkey, which ferment lactose, also give a red color.

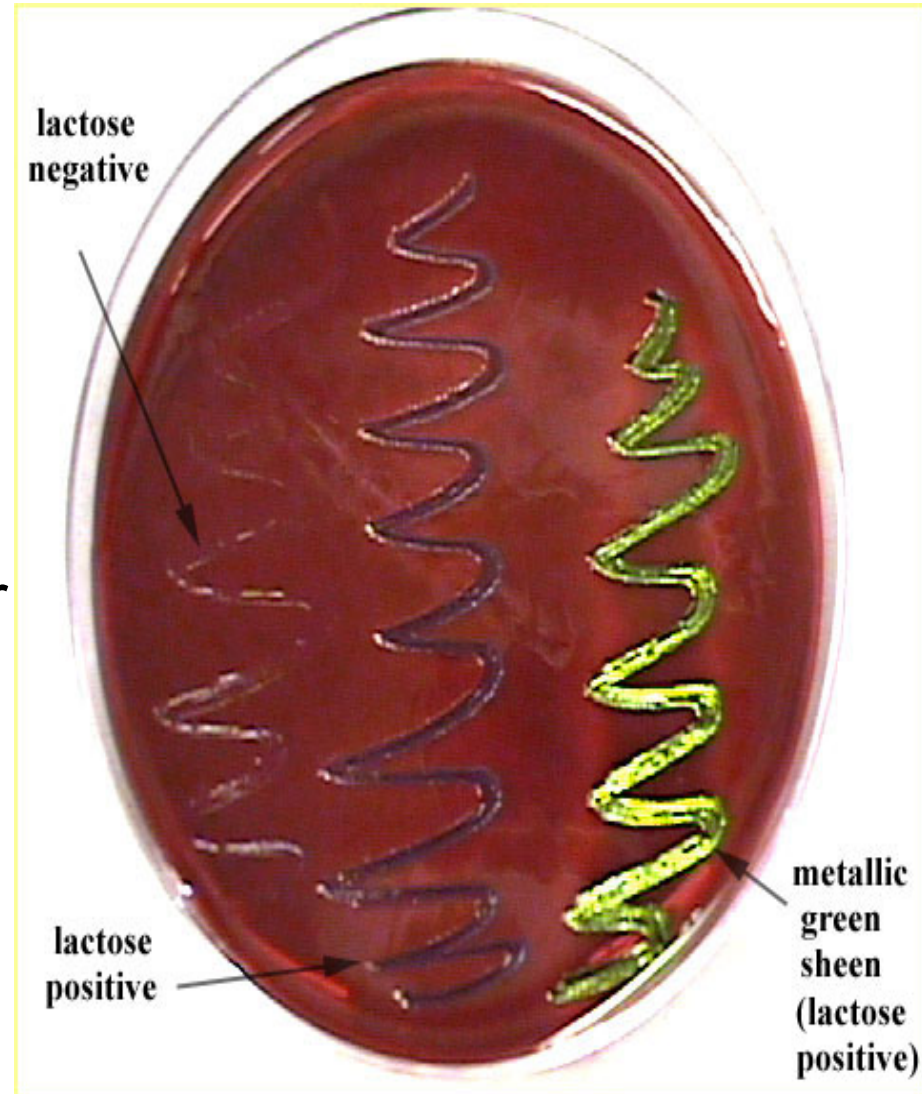
- Eosin-methylene blue (EMB) medium distinguishes organisms based on lactose fermentation.



- There are several selective and differential media used to isolate distinguishes between LF & LNF
- The most important media are:
 - MacConkey agar
 - Eosin Methylene Blue (EMB) agar
 - Salmonella Shigella (SS) agar
 - In addition to Triple Sugar Iron (TSI) agar

EMB Agar

- Bacteria that cannot ferment lactose (lactose negative) on EMB agar are transparent in color
- Those that ferment lactose (lactose positive) get a dark color
- E.coli ferments lactose and acquires a distinctive bright metallic color.

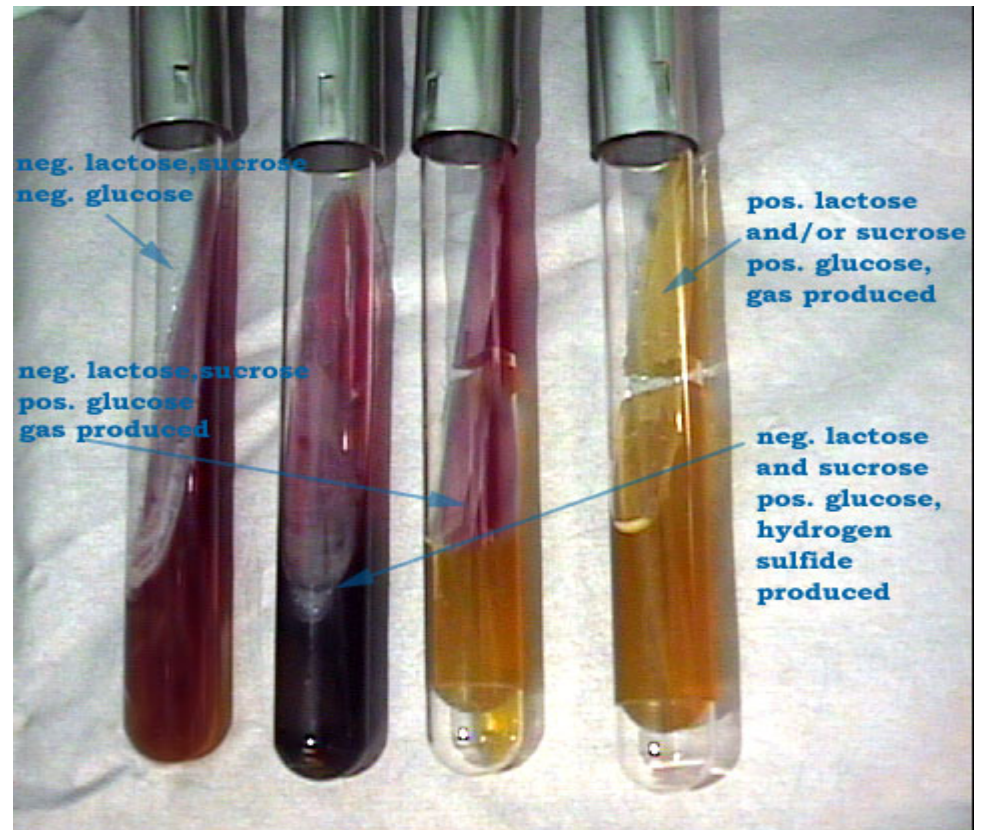


Three sugar-iron Agar (TSI)

- It is used to differentiate Enterobacteriaceae, especially *Salmonella*, from other enteric bacteria.
- Triple Sugar Iron Agar contains three carbohydrates (*glucose, lactose and sucrose*). Also contains ferrous sulfate for detection of hydrogen sulfide production
- When carbohydrates are fermented, the acid production that occurs is detected by the phenol red indicator.

- The resulting color changes are yellow for acid production and red for alkaline production. To facilitate detection of only glucose fermenting organisms, the glucose concentration is one-tenth the concentration of lactose or sucrose.
- A small amount of acid produced in the oblique part of the tube during glucose fermentation causes the medium to remain red or return to a basic pH.

- In contrast, the acid reaction (yellow) continues at the bottom of the tube, due to the low oxygen concentration at the bottom of the tube.





Pathogens Found Both Inside and Outside the Digestive Canal

- *Eschericia coli (E.coli)*

- *E. coli* ;
- To urinary tract infections
- Sepsis
- Neonatal meningitis
- Causes tourist diarrhea

General Characteristics of *E.coli*

- It is the most abundant facultative anaerobe in the large intestine and faeces.
- It ferments lactose.

Antigens:

1. 50 H antigen
2. More than 150 O antigens
3. It has 90K antigen.

Pathogenesis of *E.coli*

- The source of E. coli, which causes urinary tract infections, is the flora of the person who has colonized the urogenital region.
- The source of E.coli, which causes neonatal meningitis, is the mother's birth canal.



Intestinal Pathogens

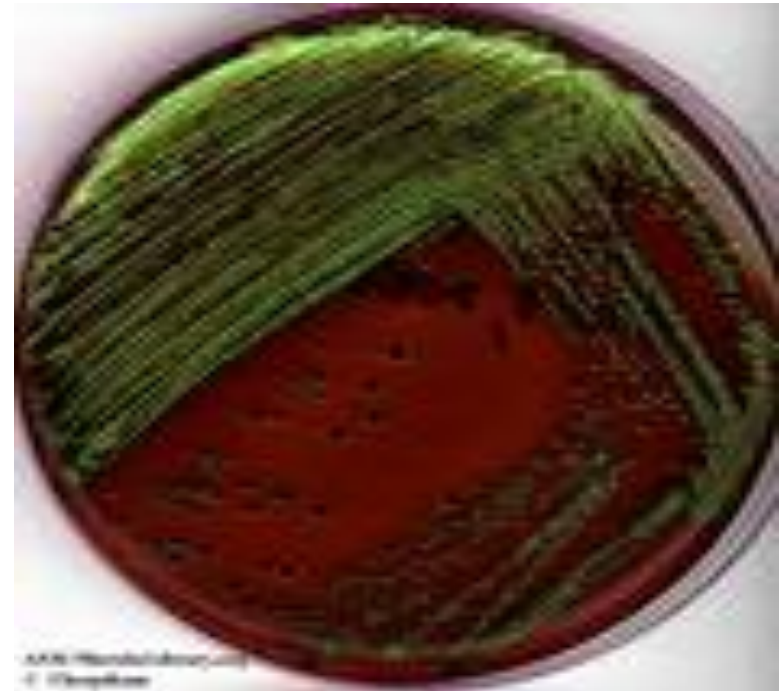
1. Enteropathogenic *E. coli* (EPEC),
 2. Enterotoxigenic *E. coli* (ETEC),
 3. Enteroinvasive *E. coli* (EIEC),
 4. Enteroaggregative *E. coli* (EAEC),
 5. Enterohemorrhagic *E. coli* (EHEC).
-

Pathogenesis of *E.coli*

- Diarrhea E.coli is transmitted through human feces, contaminated water and food.
- Enterotoxigenic E.coli; usually watery, bloodless and short-term diarrhea
- Enteropathogenic E.coli causes bloody diarrhea

Laboratory Diagnosis

- Samples are grown on media such as blood agar, EMB agar, or Mac Conkey agar.
- *E.coli* fermenting lactose takes on a **green metallic color**.



TREATMENT

- Treatment with antibiotics;

Antibiotics are selected according to

1. Localization of the disease

2. resistance of the isolate.

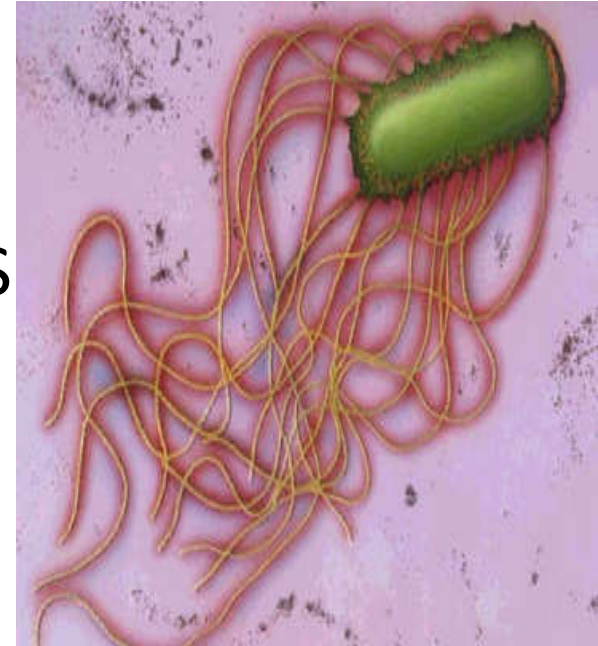
- For urinary tract infection: penicillins such as trimethoprim-sulfamethoxazole or ampicillin are used
- For sepsis: parenteral antibiotic therapy is applied.

Protection

- There is no specific protection
- Precautions can be taken by not using uncooked food and untreated water.
- In hospitals, it can be prevented by using antibiotics prophylactically and removing the catheters as soon as they are finished.

Salmonella

- non-fermenting lactose and
- Gram-negative bacilli that produce H_2S
- They cause enterocolitis, typhoid and septicemia.



Pathogenesis and Epidemiology

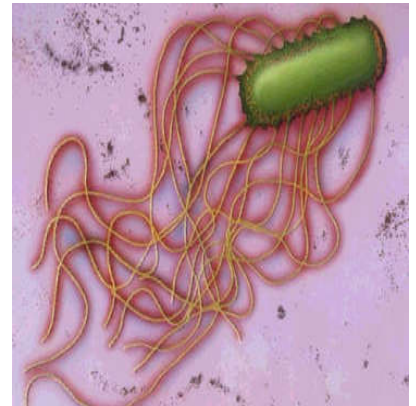
1. **Enterocolitis;** It causes inflammation and diarrhea by occupying the epithelial and subepithelial tissues of the small and large intestines.
2. **Typhoid;** It starts in the small intestine and spreads to the liver, gallbladder and spleen. It causes fever and bacteremia.
3. **Septicemia; is seen in children with** sickle cell anemia, enterocolitis and cancer.

Epidemiology

- *Salmonella*; It is transmitted by food and water in contact with human and animal residues.
- Typhoid (*Salmonella typhi*) is transmitted only from humans



1. The most common animal source:
2. Poultry,
3. Egg
4. Undercooked meat products



Clinical Findings

- Enterocolitis causes nausea, vomiting, followed by bloody and bloodless diarrhea and abdominal pain after a 12-48 hour incubation period.
- The onset of typhoid and paratyphoid diseases is slow, initially with fever and constipation.
- After the first week, high fever, loss of consciousness, enlarged spleen, leukopenia and anemia are seen.
- Intestinal bleeding and perforation occur after the 3rd week

Typhoid Symptoms

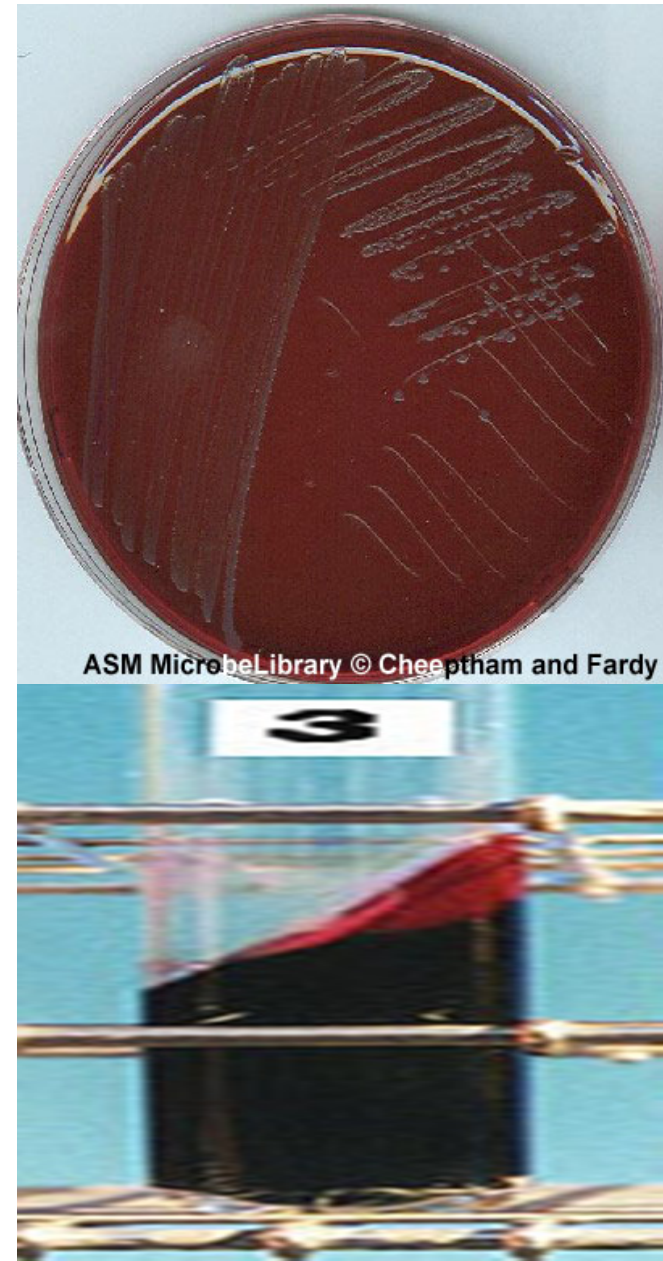
- fatigue, headache, **diarrhea** or constipation,
- fever rise,
- decrease in heart rate, **decrease in blood pressure**,
- red spots that appear on the abdomen and chest a few days after the onset of the disease,
- nose and intestinal bleeding,
- loss of appetite and weight loss,
- stomach ache,
- also in some cases, **enlargement of the spleen**,

Clinical findings-Continued

- **Septicemia**; starts with fever
- Symptoms are often seen in organs such as bones, lungs and meninges.

Laboratory Diagnosis

- Culture from stool sample or blood
- Salmonella grow colonies on MacConkey or EMB agar that do not ferment lactose (colorless).
- In TSI (Three Sugar-Iron) agar, gas and H₂S' formation is seen.



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Treatment and Prevention

- Septicemia caused by enterocolitis

1. ceftriaxone or

2. Treatment with ciprofloxacin

- for protection
- Public health and personal hygiene measures are necessary.



Pathogens in the Digestive Canal

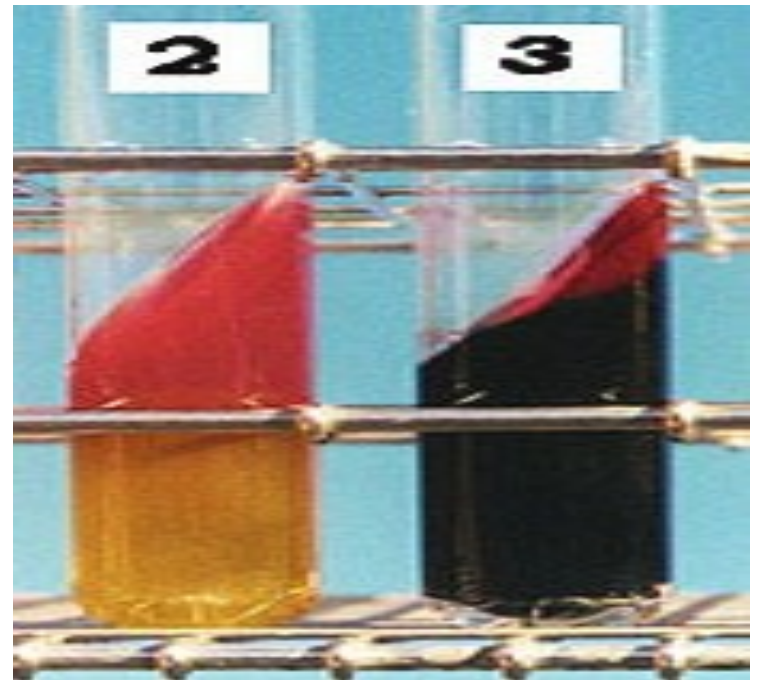
SHIGELLA

- *Shigella* species are gram-negative bacilli that do not ferment lactose.
- It causes shigellosis (dysentery).
- O antigens are found on the cell wall



Shigella

- It differs from *Salmonella* with 3 features;
1. Does not produce gas in glucose fermentation
 2. Does not produce H_2S
 3. It is motionless.



Shigella - Salmonella

Pathogenesis and Epidemiology

- Shigellosis is transmitted by the fecal-oral route.
- Infection; Unwashed hands occur through food.
- Shigella, which cause disease only in the digestive tract, invade the mucosa of the distal ileum and colon, causing bloody diarrhea (dysentery).

Clinical Findings

- After an incubation period of 1-4 days, symptoms begin with fever and abdominal cramps, followed by diarrhea with blood and mucus.
- Antibiotics can shorten the duration of the disease.



Laboratory Diagnosis

- *Diagnosis of Shigella;*

1. Makes lactose non-fermenting (colorless) colonies on MacConkey or EMB agar.
2. In three sugars - iron agar, it forms alkali in the curved part and acid in the steep part, there is no formation of gas and H₂S.
3. Slide agglutination is done
4. Neutrophil staining with methylene blue in stool



Treatment and Prevention

- The main treatment for shigellosis is to prevent the loss of fluid and electrolytes and to restore them to an adequate level.
- Antibiotic testing is mandatory.
- For protection;
 1. proper treatment of sewage waste,
 2. chlorination of water
 3. personal hygiene is necessary

VIBRIO

- Its main pathogen, *Vibrio cholerae*, is the causative agent of cholera.
- Vibrios are comma-shaped gram-negative bacilli.
- *V. cholerae* are differentiated according to O cell wall antigen
 1. O1 group members cause epidemic disease
 2. Non-O1 organisms are not pathogenic



Vibrio cholerae

1. Water
 2. foods
 3. transmitted by human-induced faecal contamination.
- The main animal stores are sea creatures such as mussels and oysters.



Pathogenesis of *Vibrio cholerae*

- The most common organism is O1 *V. cholerae*
- The pathogenesis of cholera depends on the colonization of the organism in the small intestine and the secretion of an enterotoxin called cholera toxin.
- This exotoxin can produce symptoms of cholera even if *V. cholerae* is not present in the gut.

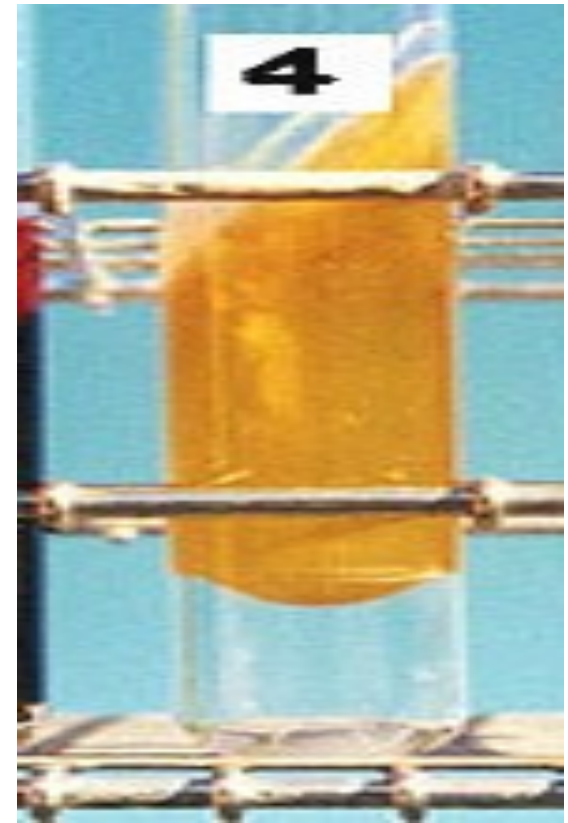
Clinical Findings

- Large volume of watery diarrhea is the main indicator of cholera.
- There are no leukocytes or erythrocyte cells in the stool.
- Loss of fluid and electrolytes leads to heart and kidney failure.



Laboratory Diagnosis

- ***Vibrio*** cholerae oxidase positive
- In TSI agar, there is no gas or H₂S output, while acid in the curved and vertical part due to the fermentation of sucrose.



Treatment and Prevention

- Fluid and electrolyte loss should be met immediately orally or intravenously.
- Antibiotics such as tetracycline are used but cannot prevent the spread of major epidemics.
- Cleanliness of water and food should be provided.
- Vaccine; Prevents the disease by 50% for 3-6 months, but cannot prevent transmission

Pathogens Outside the Digestive Canal

1. *Klebsiella*

2. *Enterobacter*

3. *Serratia Group are Pathogens*

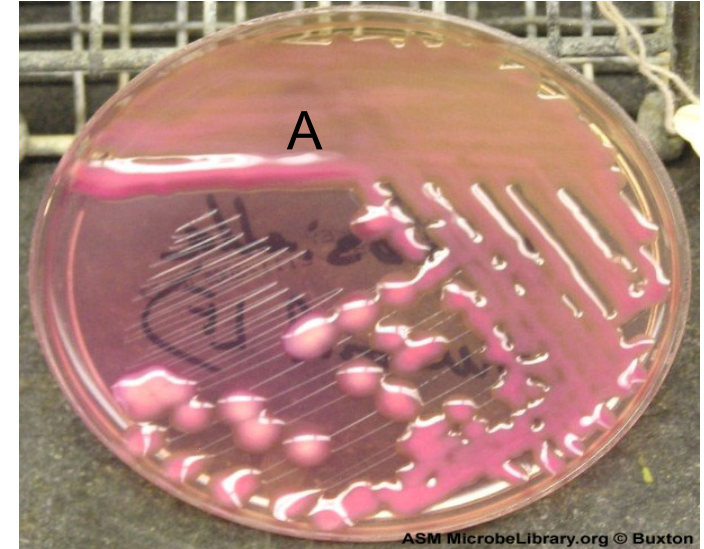
❖ They are opportunistic pathogens

❖ Causes nosocomial infections such as pneumonia and urinary tract infections.

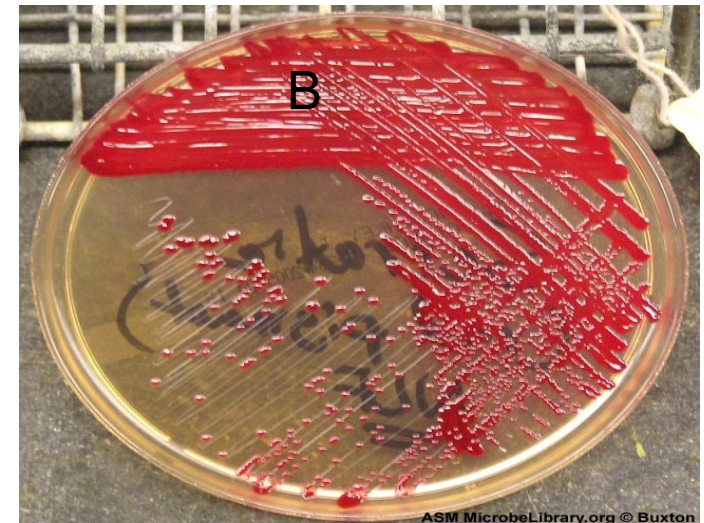
❖ It is commonly found in the large intestine, **soil and water.**

Pathogens Outside the Digestive Canal

❖ *Klebsiella pneumoniae* (A) has a very large capsule that gives its colonies a striking slimy appearance.



❖ *Serratia marcescens* (B) gives red pigmented colonies



Pathogenesis and Epidemiology

- *Klebsiella pneumoniae* is a non-opportunistic pathogen.
- It is generally seen in conditions such as advanced age, chronic respiratory failure, diabetes or alcoholism.
- *Enterobacter and Serratia* infections occur mainly in hospitalized patients

Pathogenesis and Epidemiology

- In infections caused by these organisms, except for pneumonia caused by *Klebsiella*

1. A thick and bloody sputum

2. Necrosis

3. abscess formation is observed

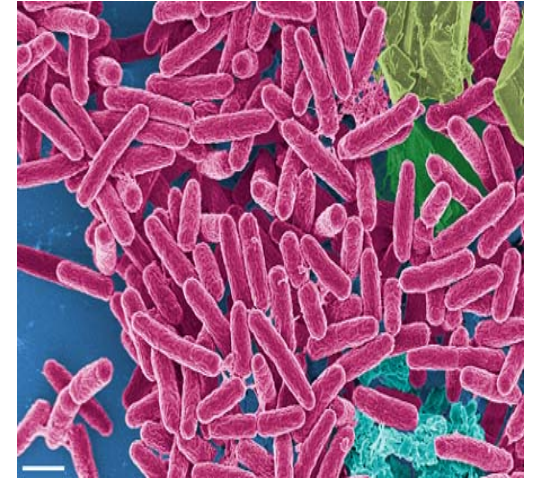
- Therefore, it is difficult to separate these organisms from each other.

Pathogenesis and Epidemiology

- Therefore, biochemical tests are used to distinguish *Klebsiella, Enterobacter, Serratia* Group Pathogens from each other.
- The choice of the drug to be used in the treatment depends on the results of the susceptibility tests.

PSEUDOMONAS

- *Pseudomonas aeruginosa* usually causes infection when host defenses are reduced.
- The incidence of *Pseudomonas cepacia* and *Pseudomonas maltophilia* is low.



PSEUDOMONAS

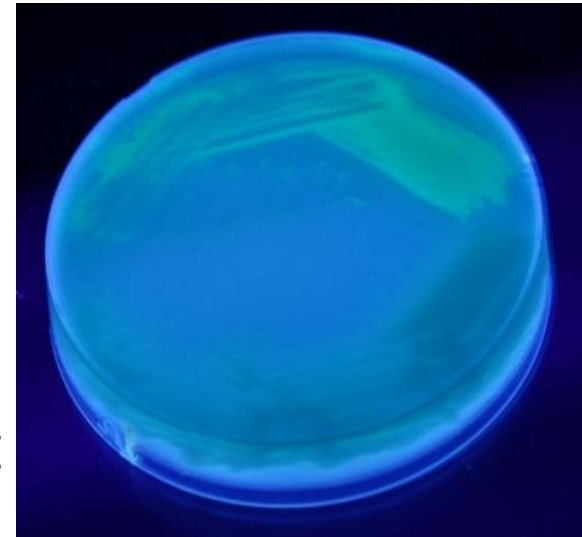
- *Pseudomonas* are aerobic gram-negative bacilli.
- They do not ferment glucose
- It is oxidase-positive
- It has the ability to reproduce in a medium containing small amounts of nutrients such as tap water.
- Resists disinfectants.

PSEUDOMONAS

➤ *Pseudomonas aeruginosa*; produces two pigments useful for diagnosis;

1. *Pyocyanin*; able to dye the pus in the wound blue
2. *Pyoverdine* is a yellow-green pigment that gives color under UV light.

➤ In the laboratory, these pigments form a blue-green color on agar.



Pathogenesis and Epidemiology

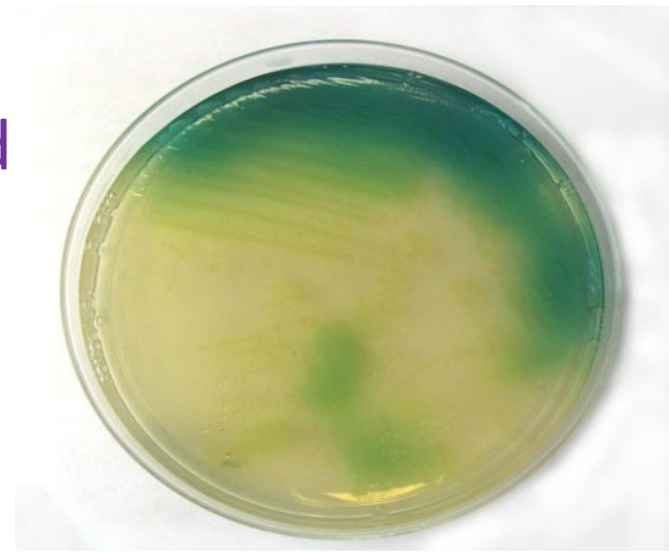
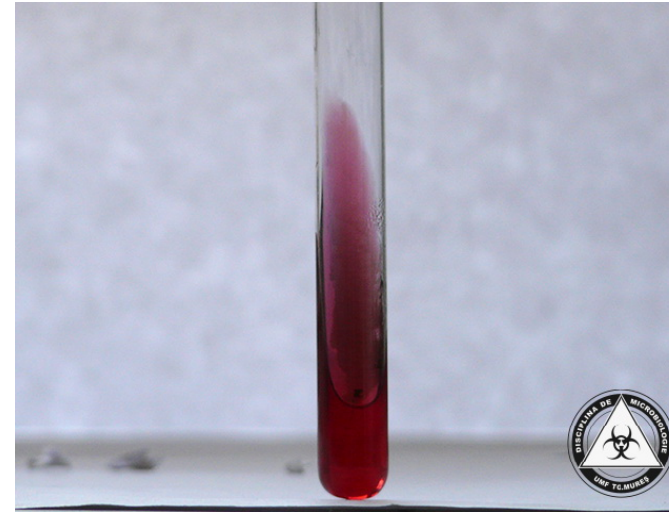
1. *Pseudomonas aeruginosa* in patients with extensive burns
2. those with chronic respiratory disease such as cystic fibrosis
3. in immunocompromised patients
4. In people whose neutrophil count is below 500/mL
5. They are opportunistic pathogens that cause infections in hospitalized patients, such as catheterized cases.

Clinical Findings

- *Pseudomonas aeruginosa* can cause urinary tract infections, pneumonia and sepsis by passing into the blood from wound infections with the endotoxins it produces.
- It spreads to the skin with its exotoxins and causes black, necrotic lesions.
- May cause corneal infections in contact lens wearers.

DIAGNOSIS

- *Pseudomonas aeruginosa*
- It produces colorless colonies on MacConkey or EMB agar.
- It is oxidase positive.
- Metallic reflection on TSI agar
- Presence of blue-green pigment and fruity odor in standard agar is used for prediagnosis.



DIAGNOSIS AND TREATMENT

- Diagnosis is determined by biochemical reactions
- Treatment
 1. ticarcillin
 2. penicillin
 3. penicillin + gentamicin
 4. Amikacin is used
- For protection, the neutrophil count should be 500/mL,
special care should be applied to the burned tissue.

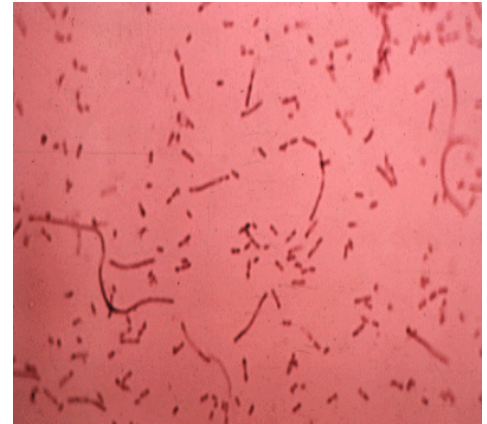
Respiratory Gram-Negative Rods

Species	Major Diseases	Laboratory Diagnosis	Factors X and V Required for Growth	Vaccine Available	Prophylaxis for Contacts
<i>H. influenzae</i>	Meningitis ¹ ; otitis media, sinusitis, pneumonia, epiglottitis	Culture; capsular polysaccharide in serum or spinal fluid	+	+	Rifampin
<i>B. pertussis</i>	Whooping cough (pertussis)	Fluorescent antibody on secretions; culture	-	+	Azithromycin
<i>L. pneumophila</i>	Pneumonia	Serology; urinary antigen; culture	-	-	None

¹ In countries where the *H. influenzae* b conjugate vaccine has been deployed, the vaccine has greatly reduced the incidence of meningitis caused by this organism.

Respiratory Gram-Negative Rods

- *HAEMOPHILUS*
- *H. influenzae* is a small gram-negative bacillus with a polysaccharide capsule.
- Serological typing is based on capsular polysaccharide antigens.
- Type b causes meningitis and sepsis
- Unencapsulated strains cause sinusitis and middle ear inflammation.



- It only infects humans.
- It enters the body through the upper respiratory tract, causing asymptomatic colonization or infections such as otitis media, sinusitis, and pneumonia.
- Ceftriaxone is used to treat *Haemophilus influenzae*.
- Diphtheria toxoid vaccines are used specifically to protect children from the disease.

Bordetella

- *Bordetella pertussis* causes whooping cough.
- *B. pertussis* are small, coccobacillus, encapsulated gram-negative bacilli.
- It is transmitted only by droplet infection of the human pathogen *Bordetella pertussis*.
- The organism attaches to the ciliary epithelium of the upper respiratory tract, followed by the death of epithelial cells

Bordetella Pathogenesis

- Pertussis symptoms:
- mild upper respiratory tract discomfort
- Paroxysmal cough lasting 1-4 weeks
- **Lymphocytosis is seen** in which the lymphocyte is increased to 70%



Bordetella Diagnosis

- The organism is isolated from the nasopharyngeal swab taken during the paroxysmal stage.
- **Bordet-Gengou medium** with a high blood content is used for isolation.

Bordetella – Typing, Prevention and Treatment

- **Typing;** by agglutination with antiserum or staining with fluorescent antibody
- **Protection;** For this, a cell-free vaccine containing 5 antigens purified from the organism or a vaccine consisting of inactivated organism are used.
- **Treatment;** erythromycin reduces the number of organisms

Legionella

- Legionella are gram-negative bacilli that stain poorly with gram stain.
- It causes pneumonia.
- Legionella mainly settle in ambient water sources such as ventilation system and water cooler dispensers.
- The entrance gate is the respiratory tract and pathological changes are seen in the lung.

Gram-negative bacilli of animal origin

- These organisms, which are transmitted from animals, cause disease in humans.
- In this way, diseases that exist in animals but can also be transmitted to humans are called zoonotic diseases.



Gram-negative bacilli of animal origin

Species	Disease	Source of Human Inflection	Mode of Transmission from Animal to Human	Diagnosis
<i>Brucella</i> species	Brucellosis	Pigs, cattle, goats, sheep	Dairy products; contact with animal tissues	Serology or culture
<i>Francisella tularensis</i>	Tularemia	Rabbits, deer, ticks	Contact with animal tissues; ticks	Serology
<i>Yersinia pestis</i>	Plague	Rodents	Flea bite	Immunofluorescence or culture
<i>Pasteurella multocida</i>	Cellulitis	Cats, dogs	Cat or dog bite	Wound culture
<i>Bartonella henselae</i>	Cat-scratch disease and bacillary angiomatosis	Cats	Cat scratch or bite; bite of cat flea	Serology or Warthin-Starry silver stain of tissue

Brucella

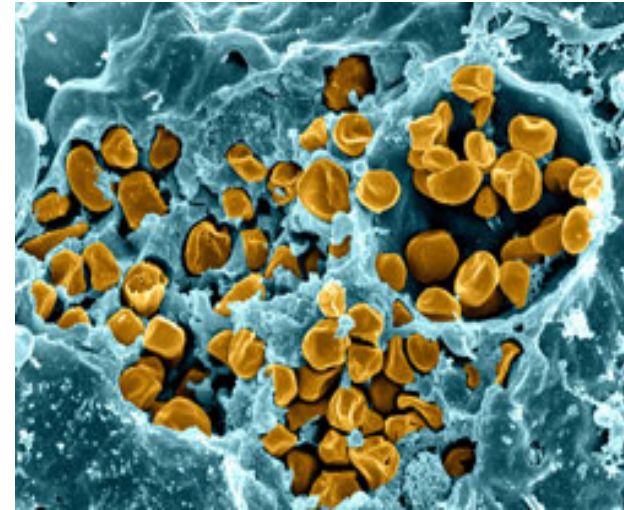
- causes brucellosis
- The organism is transmitted to the body either from contaminated milk and dairy products or through the skin by direct contact in a workplace such as a slaughterhouse.
- It settles in lymph nodes, liver, spleen and bone marrow

Brucella Diagnosis, Treatment and Prevention

- For diagnosis; Pre-typing is done by slide agglutination test with *Brucella* anti-serum.
- The organism is isolated from the serum sample taken from the patient by antibody titer.
- In the treatment, rifampin is used in addition to tetracycline.
- Vaccination of animals and milk pasteurization are important for protection.
- There is no vaccine for humans.

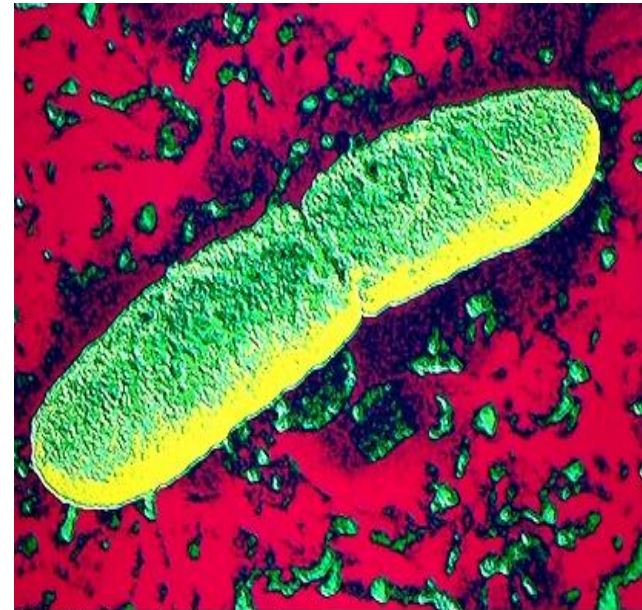
Franciella tularensis

- *F.tularensis* is a gram-negative bacillus with a single serotype.
- It is the causative agent of tularemia.
- Streptomycin is used in its treatment.
- Avoid touching ticks and wild animals.



Yersinia pestis

- Is the cause of the plague
- Small gram-negative bacilli with bipolar staining
- Mice are first infected with these bacteria by the bite of a kind of flea.
- Bacteria, which are then passed on to humans who come into contact with mice, cause swelling in the lymph nodes.



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Yersinia pestis

- Clinical manifestations
- pain, swelling in the lymph nodes
- high fever
- myalgia
- weakness
- septic shock
- pneumonia



Yersinia pestis- Diagnosis-Treatment

- for diagnosis
 1. smear or culture of pus from blood or bubo (swollen lymph nodes)
 2. fluorescent antibody staining
- For treatment; streptomycin used alone or in combination with tetracycline

Yersinia pestis- Protection

- The spread of rats in big cities should be controlled and dead wild rodents should not be touched.
- A vaccine consisting of formalin-killed organisms provides partial protection.