Lab – 5

Diagnosis of pathogenic bacteria

Gastrointestinal tract infection (Gastroenteritis / Diarrhea)

Gastroenteritis, also known as infectious diarrhea, is inflammation of the gastrointestinal tract that involves the stomach and small intestine. Signs and symptoms include some combination of diarrhea, vomiting, and abdominal pain. Fever, lack of energy, and dehydration may also occur.

Gastroenteritis can be due to infections by viruses, bacteria, parasites, and fungus. The most common cause is viruses. In children rotavirus is the most common cause of severe disease. In adults, norovirus and *Campylobacter* are common. Transmission may occur due to eating improperly prepared foods, drinking contaminated water, or through close contact with an individual who is infected.

Bacterial

In the developed world *Campylobacter jejuni* is the primary cause of bacterial gastroenteritis, with half of these cases associated with exposure to poultry. In children, bacteria are the cause in about 15% of cases, with the most common types being *Escherichia coli*, *Salmonella*, *Shigella*, and *Campylobacter* species. If food becomes contaminated with bacteria and remains at room temperature for a period of several hours, the bacteria multiply and increase the risk of infection in those who consume the food. Some foods commonly associated with illness include raw or undercooked meat, poultry, seafood, and eggs; raw sprouts; unpasteurized milk and soft cheeses; and fruit and vegetable juices. In the developing world, especially sub-Saharan Africa and Asia, cholera is a common cause of gastroenteritis. This infection is usually transmitted by contaminated water or food.

Toxigenic *Clostridium difficile* is an important cause of diarrhea that occurs more often in the elderly. Infants can carry these bacteria without developing symptoms. It is a common cause of diarrhea in those who are hospitalized and is frequently associated with antibiotic use. *Staphylococcus aureus* infectious diarrhea may also occur in those who have used antibiotics. Acute "traveler's diarrhea" is usually a

type of bacterial gastroenteritis, while the persistent form is usually parasitic. Acid-suppressing medication appears to increase the risk of significant infection after exposure to a number of organisms, including *Clostridium difficile*, *Salmonella*, and *Campylobacter* species.

Stool Analysis

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract . These conditions can include infection (such as from parasites, viruses, or bacteria), poor nutrient absorption, or cancer.

For a stool analysis, a stool sample is collected in a clean container and then sent to the laboratory. Laboratory analysis includes microscopic examination, chemical tests, and microbiologic tests. The stool will be checked for color, consistency, amount, shape, odor, and the presence of mucus. The stool may be examined for hidden (occult) blood, fat, meat fibers, bile, white blood cells , and sugars called reducing substances. The pH of the stool also may be measured. A stool culture is done to find out if bacteria may be causing an infection.

Collecting a stool sample

1-Label the container with name, date of birth and the date.

2-Place something in the toilet to catch the stool, such as a potty or an empty plastic food container, or spread clean newspaper or plastic wrap over the rim of the toilet.

3-Make sure the sample doesn't touch the inside of the toilet.

4-Use the spoon or spatula that comes with the container to 5-place the sample in a clean screw-top container and screw the lid shut.

6-If you've been given a container, aim to fill around a third of it – that's about the size of a walnut if you're using your own container.

7-Put anything you used to collect the sample in a plastic bag, tie it up and put it the bin.

8-Try not to collect urine or water from the toilet with the stool sample. If you need to urinate, do this first before collecting the stool sample.

9-Wash your hands thoroughly with soap and warm running water.

Note: A rectal swab is sometimes considered an easy, viable alternative to a stool sample.

The stool collection process can be more difficult with infants in diapers or people with active diarrhea. If collecting a stool sample from baby, use a cotton swab to collect a sample from rectum.

Stool culture

A stool culture is a test on a stool sample to find germs (such as bacteria or a fungus) that can cause an infection. A sample of stool is added to a substance that promotes the growth of germs. If no germs grow, the culture is negative. If germs that can cause infection grow, the culture is positive. The type of germ may be identified using a microscope or chemical tests. Sometimes other tests are done to find the right medicine for treating the infection. This is called sensitivity testing.

A stool culture is done to:

- **Find the cause of symptoms. It can help explain symptoms such as severe or bloody diarrhea or an increased amount of gas. It can also help find the cause of nausea, vomiting, loss of appetite, bloating, belly pain and cramping, and fever.**
- **Find and identify certain types of organisms that are causing infections or diseases. These include food poisoning, inflammation of the large intestine (colitis), cholera, and typhoid.**
- **Identify a person who may not have any symptoms of disease but who carries bacteria that can spread infection to others. This person is called a carrier. A person who is a carrier and who handles food is likely to infect others.**
- **Find out if treatment for an infection has worked as it should.**

Stool samples contain

Stools contain bacteria and other substances that are present in the digestive system.

By testing the levels of these substances and bacteria in the stools, it's possible to work out what's happening in digestive system.

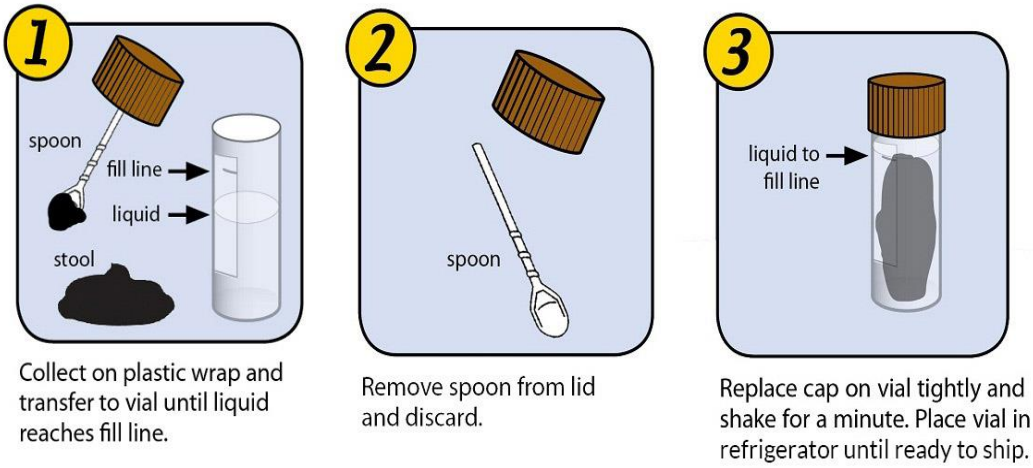
Storing a stool sample

Stool samples should be handed in as soon as possible, as some can't be analysed if they've been refrigerated.

If you can't hand the stool sample in immediately, you should store it in a fridge, but for no longer than 24 hours. Place the container in a sealed plastic bag first.

Stool samples must be fresh – if they aren't, the bacteria in them can multiply. This means the levels of bacteria in the stool sample won't be the same as the levels of bacteria in digestive system. If the levels of bacteria don't match, the test results may not be accurate.

*Container for stool sample collection / *Swabs for stool sample collection



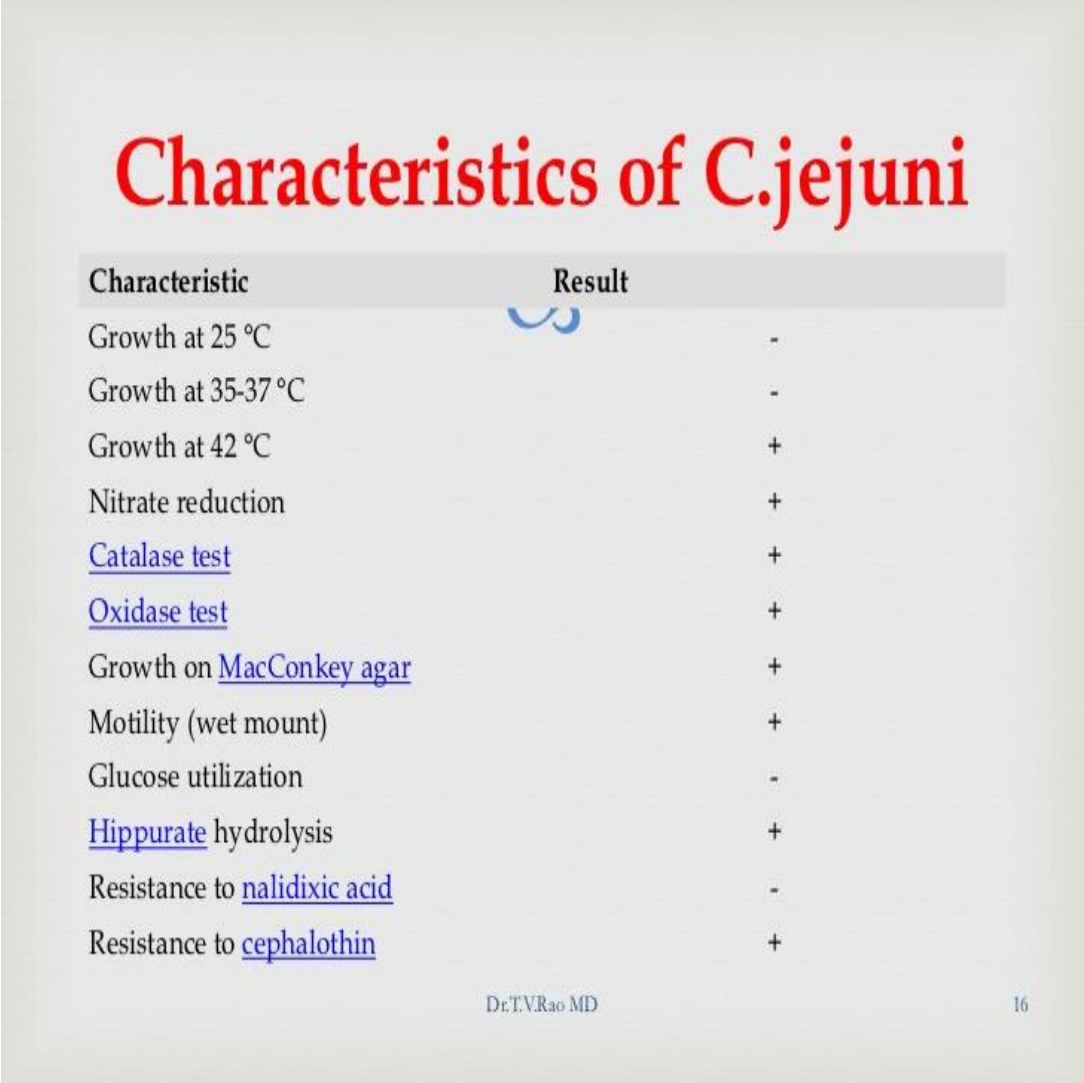
Cause of Gastroenteritis

*Campylobacter spp

Campylobacter **(meaning "curved bacteria") is a genus of Gram-negative bacteria , family Campylobacteraceae. *Campylobacter* typically appear comma or S-shaped and motile by unipolar or bipolar flagella. They generally survive in environments with low oxygen (Microaerophilic). They are positive by the oxidase test and catalase test. *Campylobacter* are nonfermentative. *Campylobacters* are best cultured at 42 °C .Survival at room temperature is poor, but *Campylobacters* can survive for a short time at refrigeration temperatures – up to 15 times longer at 2°C than at 20°C. The bacteria die out slowly at freezing temperatures and is heat sensitive, the cells are destroyed at temperatures above 48°C.**

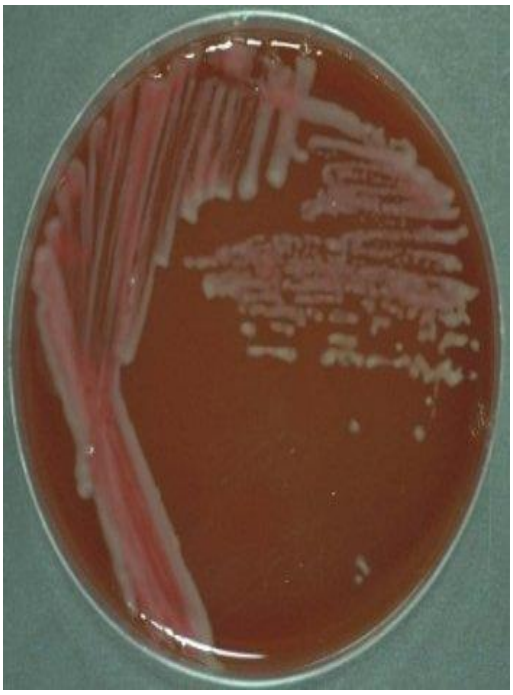
Most *Campylobacter* species can cause disease and can infect humans and other animals. The bacterium's main reservoir is poultry; humans can contract the disease from eating food contaminated with *Campylobacter* species. Another source of infection is contact with infected animals, which often carry *Campylobacter* asymptomatically. At least a dozen species of *Campylobacter* have been implicated in human disease, with *C. jejuni* and *C. coli* being the most common. *C. jejuni* is now recognized as one of the main causes of bacterial foodborne disease in many developed countries. *C. jejuni* infection can also spread to the blood in individuals with AIDS, while *C. lari* is a known cause of recurrent diarrhea in children. *C. fetus* is a cause of

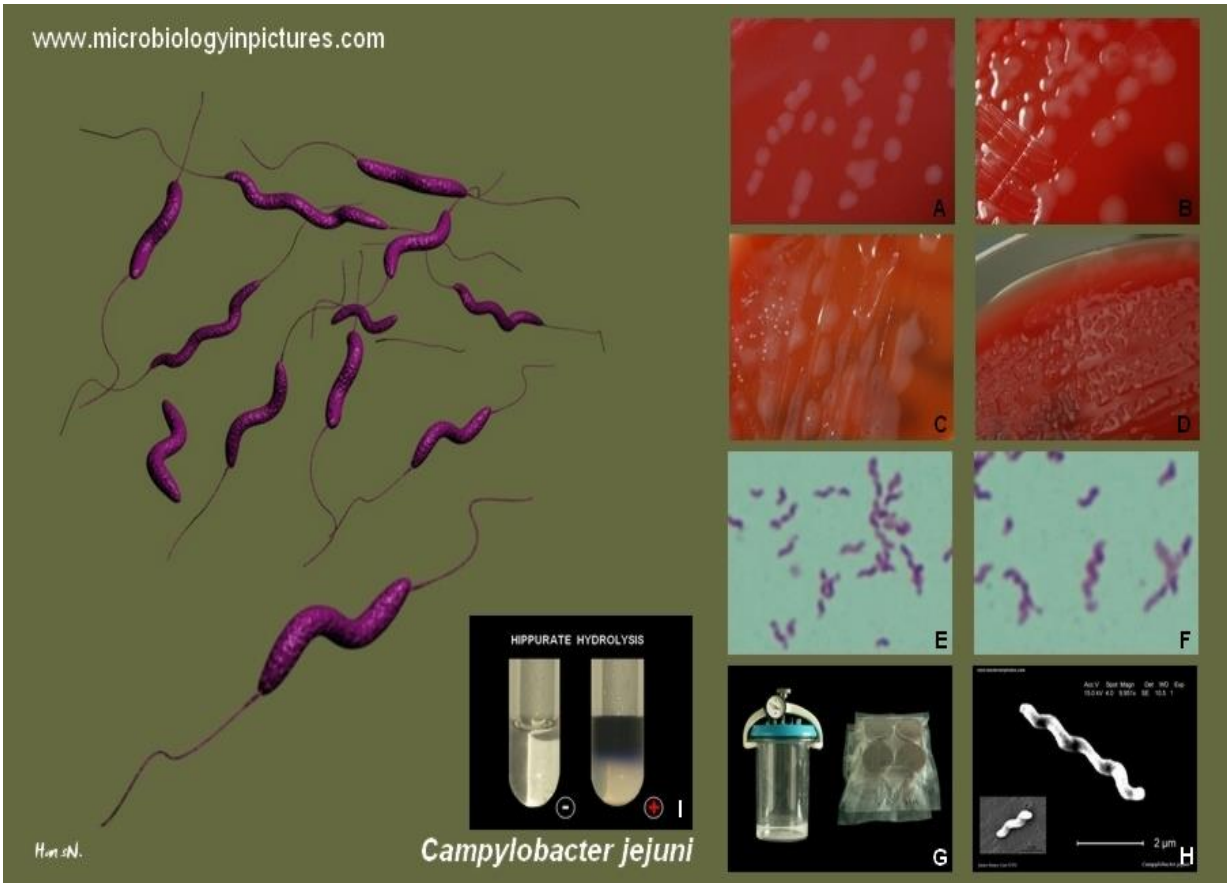
spontaneous abortions in cattle and sheep, as well as an opportunistic pathogen in humans.



campylobacter jejuni

- Campylobacter spp. are small (0.5–8 µm long × 0.2–0.5 µm wide).
- motile,
- non–spore-forming,
- curved (comma-shaped), (**seagull wing shaped**) or S-shaped gram-negative bacilli .
- grow optimally in an atmosphere containing 5%–10% oxygen and, therefore, are considered to be **microaerophilic**.
- A single stool specimen is generally adequate to detect Campylobacter spp .





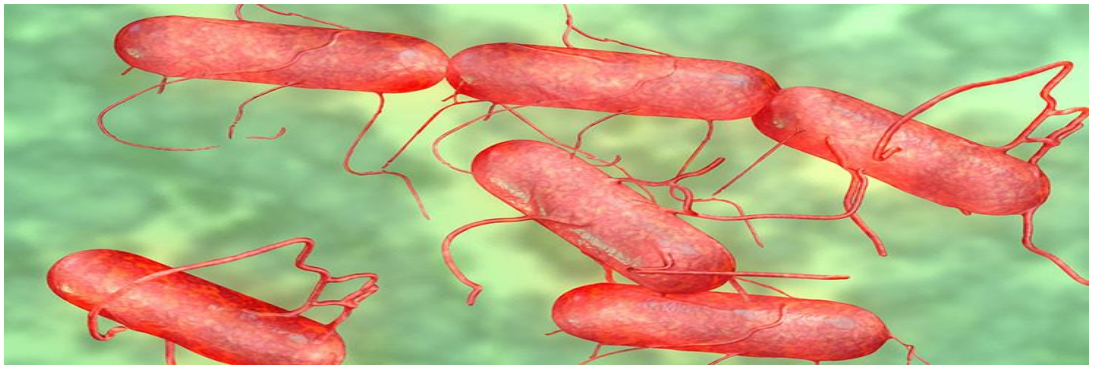
*Salmonella spp

***Salmonella* is a genus of rod-shaped (bacillus) gram-negative bacteria of the Enterobacteriaceae family. The two species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is the type species and is further divided into six subspecies that include over 2,500 serotypes. Strains of *Salmonella* cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning (salmonellosis). *Salmonella* species are nonspore-forming, predominantly motile enterobacteria with cell diameters between about 0.7 and 1.5 µm, lengths from 2 to 5 µm, and peritrichous flagella (all around the cell body).. They are also facultative anaerobes, capable of surviving with or without oxygen. Most subspecies of *Salmonella* produce hydrogen sulfide, which can readily be detected by growing them on media containing ferrous sulfate, such as is used in the triple sugar iron test.**

The bacteria are not destroyed by freezing, but UV light and heat accelerate their destruction. They perish after being heated to 55 °C (131 °F) for 90 min, or to 60 °C (140 °F) for 12 min. To protect against

***Salmonella* infection, heating food for at least 10 minutes to an internal temperature of 75 °C (167 °F) is recommended.**

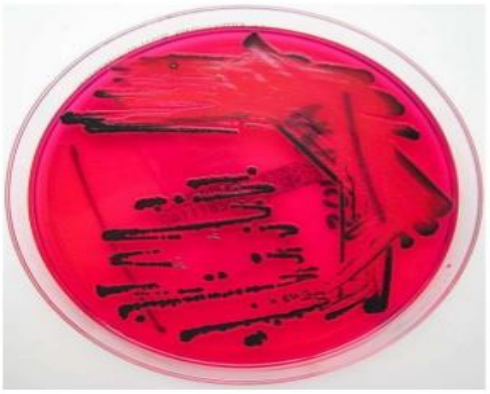
***Salmonella* species can be found in the digestive tracts of humans and animals, especially reptiles. *Salmonella* on the skin of reptiles or amphibians can be passed to people who handle the animals. Food and water can also be contaminated with the bacteria if they come in contact with the feces of infected people or animals.**



Salmonella enterica colonies growing on XLD Agar Incubated aerobically for 24 hours at 35°C

On Xylose lysine deoxycholate agar

- **Salmonella** metabolise thiosulfate to produce hydrogen sulfide, which leads to the formation of colonies with black centers and allows them to be differentiated from the similarly coloured ***Shigella*** colonies.



Reaction on Salmonella Shigella (SS) agar

- SS agar is a selective & differential medium used for isolation of *Salmonella* and *Shigella*
- The selective agents are **bile salts**, and **brilliant green dye**, which inhibit gram-positive organisms
- The medium contains only **lactose** as a differential agent and thus differentiates on the basis of lactose fermentation
- The formation of acid on fermentation of lactose causes the neutral red indicator to make pink colonies
- Non lactose fermenting organisms are colorless on the medium
- SS agar contains **sodium thiosulfate** and **ferric ammonium citrate** allows the differentiation of organisms that produce H2S
  - Lactose fermenters, such as *E. coli*, have colonies which are pink
  - *Shigella* appears transparent or amber
  - *Salmonella* appears transparent or amber with black centers due to H2S production

Lactose —(Lactose fermenter)→ Acid —(Neutral red)→ Pink colonies

H2S + Ferric ammonium citrate → Ferrous sulfide Black precipitate

Biochemical Test and Identification of *Salmonella. spp*

Characteristics	*Salmonella*. spp
Capsule	Negative (-ve)
Catalase	Positive (+ve)
Citrate	Positive (+ve) except S.typhi(-ve)
Flagella	Positive (+ve)
Gas	Negative (-ve)
Gelatin Hydrolysis	Negative (-ve)
Gram Staining	Negative (-ve)
H2S	Positive (+ve)
Indole	Negative (-ve)
Motility	Motile except *S. gallinarum*
MR (Methyl Red)	Positive (+ve)
Nitrate Reduction	Positive (+ve)
Oxidase	Negative (-ve)
Pigment	Negative (-ve)
Shape	Rod
Spore	Negative (-ve)
TSIA (Triple Sugar Iron Agar)	Alkaline/Acid
Urease	Negative (-ve)
VP (Voges Proskauer)	Negative (-ve)
Fermentation of	
Lactose	Negative (-ve)
Mannitol	Positive (+ve)
Sucrose	Negative (-ve)
Xylose	Positive (+ve)

*Shigella spp

***Shigella* is a genus of Gram-negative, facultative anaerobic, nonspore-forming, nonmotile, rod-shaped bacteria genetically closely related to *E. coli*. The genus is named after Kiyoshi Shiga, who first discovered it in 1897.**

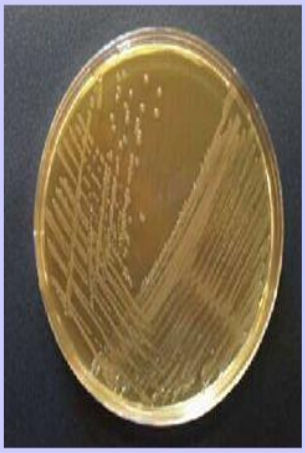
The causative agent of human shigellosis, *Shigella* species are classified by four serogroups:

- **Serogroup *A*: *S. dysenteriae* (15 serotypes)**
- **Serogroup *B*: *S. flexneri* (six serotypes)**
- **Serogroup *C*: *S. boydii* (19 serotypes)**
- **Serogroup *D*: *S. sonnei* (one serotype)**

The diagnosis of shigellosis is made by isolating the organism from diarrheal fecal sample cultures. *Shigella* species are negative for motility and are generally not lactose fermenters, but *S. sonnei* can ferment lactose. They typically do not produce gas from carbohydrates (with the exception of certain strains of *S. flexneri*) and tend to be overall biochemically inert. *Shigella* should also be urea hydrolysis negative. When inoculated to a triple sugar iron slant, they react as follows: K/A, gas -, and H_2S -. Indole reactions are mixed, positive and negative, with the exception of *S. sonnei*, which is always indole negative. Growth on Hektoen enteric agar produces bluish-green colonies for *Shigella* and bluish-green colonies with black centers for *Salmonella*.

Shigella spp Isolation

- *Shigella* organisms may be very difficult to distinguish biochemically from *Escherichia coli.*
- *Shigella* species are Gram-negative, facultatively anaerobic, nonsporulating, nonmotile rods in the family *Enterobacteriaceae*.
- They do not decarboxylate lysine or ferment lactose within 2 days.



NON-LACTOSE FERMENTERS: COLOURLESS COLONIES

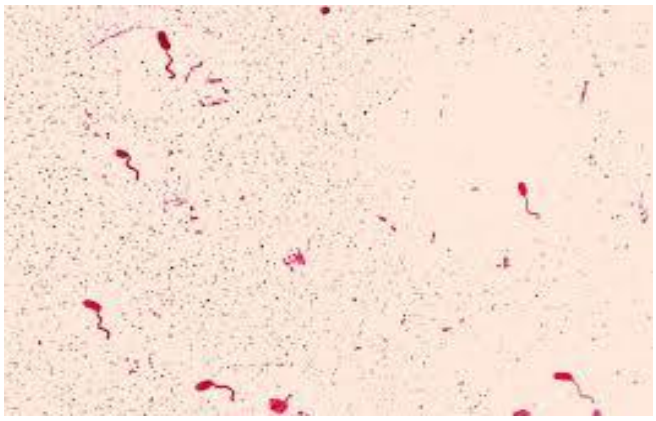
MacConkey Agar
LACTOSE FERMENTERS: RED/PINK COLONIES

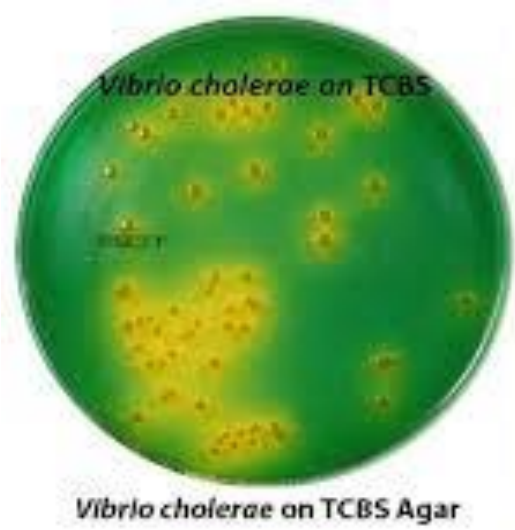
Biochemical Test and Identification of *Shigella .spp*

Characteristics	*Shigella* .spp
Capsule	Negative (-ve)
Catalase	Positive (+ve)
Citrate	Negative (-ve)
Flagella	Negative (-ve)
Gas	Negative (-ve)
Gelatin Hydrolysis	Negative (-ve)
Gram Staining	Negative (-ve)
H2S	Negative (-ve)
Indole	Variable except *Sh.sonnei* (-ve)
Motility	Non-Motile
MR (Methyl Red)	Positive (+ve)
Oxidase	Negative (-ve)
Pigment	Negative (-ve)
Shape	coccobacilli
Spore	Negative (-ve)
TSIA (Triple Sugar Iron Agar)	Alkaline /Acid
Urease	Negative (-ve)
VP (Voges Proskauer)	Negative (-ve)
Fermentation of	
Glucose	+ve no gas except *Sh.flexneri*(-ve)
Lactose	Negative (-ve) *Sh.sonnei* (+ve)
Mannitol	Variable
Sucrose	Negative (-ve)
Xylose	Negative (-ve)

*Vibrio spp

***Vibrio* is a genus of Gram-negative bacteria, possessing a curved-rod shape (comma shape), several species of which can cause foodborne infection, usually associated with eating undercooked seafood. Typically found in salt water, *Vibrio* species are facultative anaerobes that test positive for oxidase and do not form spores. All members of the genus are motile and have polar flagella with sheaths. Pathogenic *Vibrio* species include *V. cholerae* (the causative agent of cholera), *V. parahaemolyticus*, and *V. vulnificus*. *V. cholerae* is generally transmitted by contaminated water.**





Vibrio cholerae on TCBS Agar

Vibrio parahaemolyticus on TCBS Agar

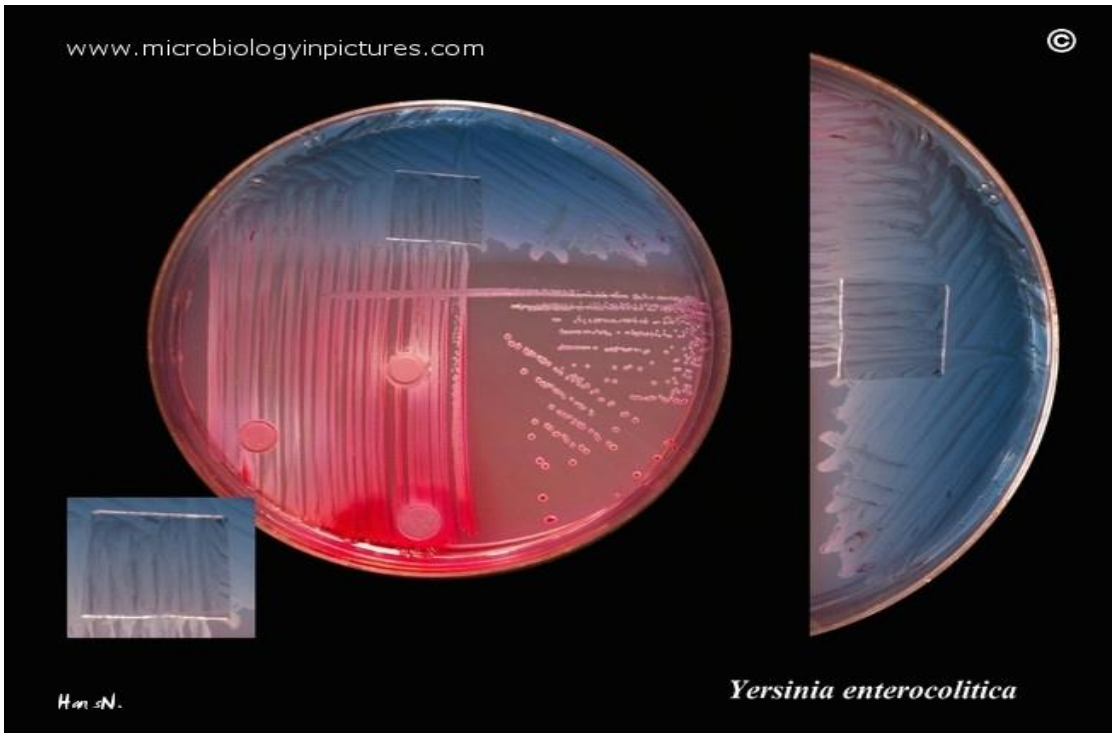
Biochemical Test and Identification of *Vibrio cholerae*

Basic Characteristics	Properties (*Vibrio cholerae*)
Capsule	Non-Capsulated
Citrate	+ve
Flagella	Flagellated
Gas	-ve
Gelatin Hydrolysis	+ve
Gram Staining	-ve
H2S	-ve
Hemolysis	Beta Hemolysis
Indole	+ve
Motility	Motile
MR (Methyl Red)	-ve
Nitrate Reduction	+ve
Oxidase	+ve
Shape	Rods
Spore	Non-Sporing
String Test	+ve
Urease	-ve
VP (Voges Proskauer)	Variable
Fermentation of	
Lactose	Variable
Mannitol	+ve
Sucrose	+ve
Xylose	-ve

*Yersinia entercolitica

***Yersinia enterocolitica* is a Gram-negative bacillus-shaped bacterium, belonging to the family Enterobacteriaceae. It is motile at temperatures of 22–29°C, but becomes nonmotile at normal temperature. The genus *Yersinia* includes 11 species: *Y. pestis, Y. pseudotuberculosis, Y. enterocolitica, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. bercovieri, Y. mollaretii, Y. rowdies, Y. aldovae,* and *Y. ruckeri*. Among them, only *Y. pestis, Y. pseudotuberculosis,* and certain strains of *Y. enterocolitica* are of pathogenic importance for humans.**

Human pathogenic strains are usually confined to the intestinal tract and lead to enteritis/diarrhea.



Typically positive results with *Yersinia enterocolitica*:

- **Ornithine decarboxylase**
- **Urease test** (about 25% negative)
- **ONPG**
- **Cellobiose**

- **Sucrose**
- **Mannitol**
- **Methyl red**
- **Motility at 25°C**

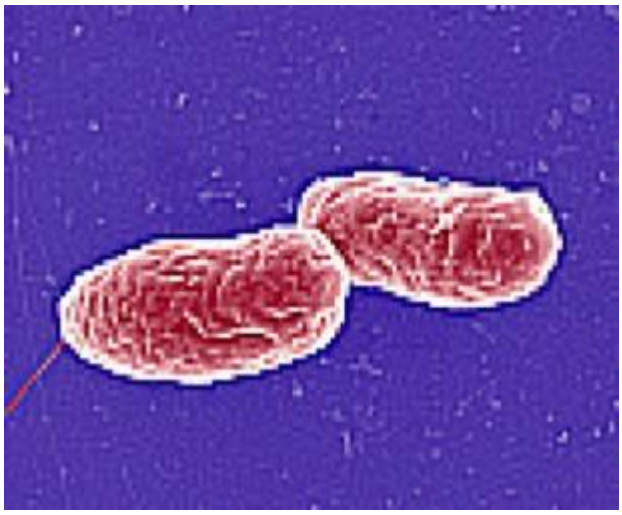
Typically negative results with *Yersinia enterocolitica*:

- **Hydrogen sulfide**
- **Lysine decarboxylase**
- **Arginine dihydrolase**
- **Simmons citrate**
- **Voges-Proskauer test (acetoin)**; negative after 24 hours at 36°C but **positive after 48h at 25°C**
- **Phenylalanine deaminase**
- **Motility at 36°C**

Positive or negative:

- **Indole production** (about 50% positive)
- **Esculin hydrolysis** (about 25% positive)

Plesiomonas shigelloides



Plesiomonas shigelloides **is a species of bacteria that was formerly classified in the family *Vibrionaceae*, but now most microbiologists agree that a better classification is in the family Enterobacteriaceae. *P.***

shigelloides **isolated from freshwater, freshwater fish, and shellfish and from many types of animals including humans, cattle, goats, swine, cats, dogs, monkeys, vultures, snakes, and toads.**

It is a facultative anaerobic, Gram-negative bacterium. *P. shigelloides* utilizes glucose as a source of carbon as well as energy source. *P. shigelloides* is a short gram-negative rod. It is oxidase-positive and catalase-positive. The organism moves by polar flagella which are lophotrichous, meaning they have multiple flagella in the same area. Because of this, the bacteria generally move in one direction. *P. shigelloides* can cause diarrhea/gastroenteritis in humans. *Plesiomonas* can be distinguished from *Shigella* in diarrheal stools by an oxidase test: *Pleisomonas* is oxidase positive and *Shigella* is oxidase negative. *Pleisomonas* is negative for DNAse, and this and other biochemical tests distinguish it from *Aeromonas sp.*

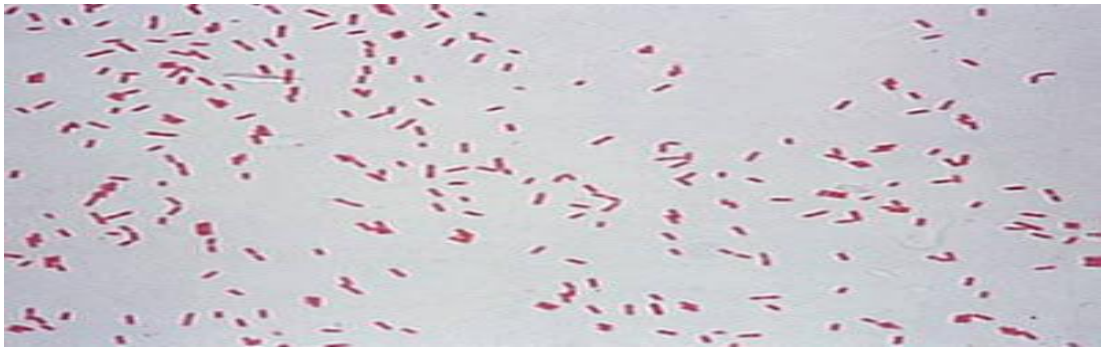
Biochemical Test and Identification of *Pleisomonas shigelloides*

Characteristics	*Pleisomonas shigelloides*
Capsule	Negative (-ve)
Catalase	Positive (+ve)
Citrate	Negative (-ve)
Flagella	Positive (+ve)
Gas from glucose	Negative (-ve)
Gelatin Hydrolysis	Negative (-ve)
Gram Staining	Negative (-ve)
H2S	Negative (-ve)
Indole	Positive (+ve)
Motility	Motile
MR (Methyl Red)	Positive (+ve)
Oxidase	Positive (+ve)
Shape	Rod
Spore	Negative (-ve)
TSIA (Triple Sugar Iron Agar)	Acid /Acid
Urease	Negative (-ve)
Blood hemolytic	Non- Blood hemolytic
Phenylalanine deaminase	variable

VP (Voges Proskauer)	Negative (-ve)
Fermentation of	
Lactose	Positive (+ve)
Mannitol	Negative (-ve)
Sucrose	Negative (-ve)
Glucose	Positive (+ve)
Inositol	Positive (+ve)
Xylose	Negative (-ve)

*Aeromonas spp

Aeromonas **is a genus of Gram-negative, facultative anaerobic, rod-shaped bacteria that morphologically resemble members of the family Enterobacteriaceae. Cells contain a single polar flagellum, so are motile. The organism tests positive for the ability to produce catalases and oxidases, and is capable of reducing nitrate to nitrite . The species is able to undergo both respiration and fermentation. Strains are able to grow on MacConkey agar with an optimal growth temperature of 30–37°C. The most important pathogens are *A. hydrophila*, *A. caviae*, and *A. veronii* biovar *sobria*. The organisms are ubiquitous in fresh and brackish water. Two major diseases associated with *Aeromonas* are gastroenteritis and wound infections, with or without bacteremia. Gastroenteritis typically occurs after the ingestion of contaminated water or food, whereas wound infections result from exposure to contaminated water. Some potential virulence factors of *Aeromonas* (e.g. endotoxins, hemolysins, enterotoxins, adherence factors).**



Aeromonas hydrophila

Biochemical Test and Identification of *Aeromonas hydrophila*

Characteristics	*Aeromonas hydrophila*
Capsule	Negative (-ve)
Catalase	Positive (+ve)
Citrate	Negative (-ve)
Flagella	Positive (+ve)
Gas	Positive (+ve)
Gelatin Hydrolysis	Positive (+ve)
Gram Staining	Negative (-ve)
H2S	Negative (-ve)
Indole	Positive (+ve)
Motility	Motile
MR (Methyl Red)	Negative (-ve)
Oxidase	Positive (+ve)
Shape	Rod
Spore	Negative (-ve)
TSIA (Triple Sugar Iron Agar)	AK /Acid or A/A
Urease	Negative (-ve)
VP (Voges Proskauer)	Positive (+ve)
Fermentation of	
Lactose	Variable(+/-)
Mannitol	Positive (+ve)
Sucrose	Positive (+ve)
Xylose	Positive (+ve)

Laboratory diagnosis

1-Specimen culture

1-Enrichment media

***Selenite F broth**

***Alkaline peptone water**

***Tetrathionate broth**

*** Campylobacter Enrichment Broth (Bolton formula, Oxoid AM7526 or Malthus Diagnostics LAB-135, Malthus Diagnostics, North Ridgeville, OH; 216-327-2585) with lysed horse blood and antibiotic supplement (Oxoid NDX131 or Malthus Diagnostics X131). Alternatively, antibiotic supplement may be prepared from individual components (G-1).**

2- Selective differential media

***MacConkey agar**

***Blood agar**

***TCBS agar**

***XLD agar**

***bAeyta-Hunt –Bark agar without antibiotics (for campylobacter)**

***Heart infusion agar (HIA) Slant (for campylobacter)**

2- Microscopy

***Gram stain**

3-Biochemical tests

- **Catalase Test**
- **Motility test**

- **Oxidase Test**
- **Methyl Red / Voges-Proskauer (MR/VP)**

- **Kliger’s Iron Agar (KIA)**
- **Nitrate Broth**
- **Simmon’s Citrate Agar**
- **Urease test**
- **Carbohydrates fermentation test**
- **Sulfur Indole Motility Media (SIM)**

4- Serological methods