Water-Born infections

Campylobacter

One of the most common cause of human bacterial gastroenteritis, with Campylobacter jejuni being the predominantly isolated species. They are widespread in the environment and occur very commonly in the intestinal tracts of animals, including birds. 95% of ready-prepared chickens are contaminated with Campylobacter, and poultry meat is thought to be an important source of infection. Wild birds also have a high intestinal colonization rate. Campylobacter can easily be isolated from surface waters.

Microscopically: curved, spiral, or S-shaped organisms that microscopically resemble vibrios. A single, **polar flagellum** provides the organism with its characteristic darting motility. polar flagella are attached at their ends giving **"gull wings" appearance**.

Culture: Skirrow´s media, Campy media, microaerophilic conditions. That is, it requires oxygen at reduced tension (5 – 10%).

There is several spp. have been associated with human diseases, of these, *C. jejuni*, and *C. coli* are the most common and similar enough to be considered as one.

Disease: Campylobacter infect the intestine and can cause ulcerative, inflammatory lesions in the jejunum, ileum, or colon.
Campylobacter are transmitted to humans primarily via the **fecal–oral route** through **direct contact**, **exposure to contaminated meat** (especially poultry), or **contaminated water** supplies.

<u>Pathogenesis and clinical significance</u>
Campylobacter may cause both intestinal and extraintestinal disease.

C. jejuni typically causes an acute enteritis in otherwise healthy individuals following a 1- to 7-day incubation. The disease lasts days to several weeks and, generally, is self-limiting. Symptoms may be both systemic (fever, headache, myalgia) and intestinal (abdominal cramping and diarrhea, which may or may not be bloody).

Important **virulence factors** include a **cytotoxin** that may be involved in inflammatory colitis and an **enterotoxin** (related to cholera toxin) that

results in increased adenylyl cyclase activity and, therefore, electrolyte and fluid imbalance.

ESCHERICHIA COLI

Escherichia coli is part of the **normal flora** of the colon in humans and other animals but can be pathogenic both within and outside of the GI tract.

The differences in the **degree of virulence** of various *E. coli* strains is correlated with the acquisition of plasmids, integrated prophages, and pathogenicity islands.

E. coli has **fimbriae or pili** that are important for adherence to host mucosal surfaces, and different strains of the organism may be **motile or nonmotile**.

Typing strains is based on differences in three structural antigens: **O, H, and K**.

Clinical significance: intestinal disease

Transmission of intestinal disease is commonly by the **fecal–oral route**, with contaminated food and water serving as vehicles for transmission. Seven such groups have been defined, of which three may be waterborne

Enteropathogenic *E. coli* have been associated with outbreaks in children in nurseries and hospital wards. These strains belong to particular "O" serotypes.

Enterotoxigenic *E. coli* are a common cause of diarrhoea in travellers. They are identified by the production of a heat-stable toxin and a heatlabile toxin.

Verocytotoxigenic *E. coli* (VTEC) cause serious diarrhoeal disease, with bloody diarrhoea and painful abdominal cramps. In 10–15% of cases, haemolytic uraemic syndrome develops as a complication, which can result in kidney failure or even death. The most frequent serotype isolated is O157, but other serotypes, such as O139, have been reported. The organism is common in cattle and sheep and other farm animals, in which it behaves as a commensal organism and does not cause any recognized disease. The infectious dose for VTEC is very low, about 10–100 organisms, which explains their potential to cause waterborne outbreaks when animal faeces-contaminated material gains access to water supplies past treatment or where treatment is inadequate.

Clinical significance: extraintestinal disease

Urinary tract infection: E. coli is the most common cause of urinary tract infection (UTI), including cystitis and pyelonephritis.

Nosocomial (hospital-acquired) infections: These include sepsis/ bacteremia, endotoxic shock, and pneumonia.

Laboratory identification

1. Intestinal disease: Because *E. coli* is normally part of the intestinal flora, detection in stool cultures of disease-causing strains is generally difficult.

Molecular techniques, such as PCR may be employed to identify E. coli strains producing Shiga-like toxins.

2. Extraintestinal disease: Isolation of E. coli from normally sterile body sites (for example, the bladder or cerebrospinal fluid) is diagnostically significant. Specimens may be cultured on MacConkey.

Prevention and treatment

Intestinal disease can be prevented by care in selection, preparation, and consumption of food and water.

Maintenance of fluid and electrolyte balance is of primary importance in treatment.

Antibiotics may shorten duration of symptoms, but resistance is nevertheless widespread.

SALMONELLA

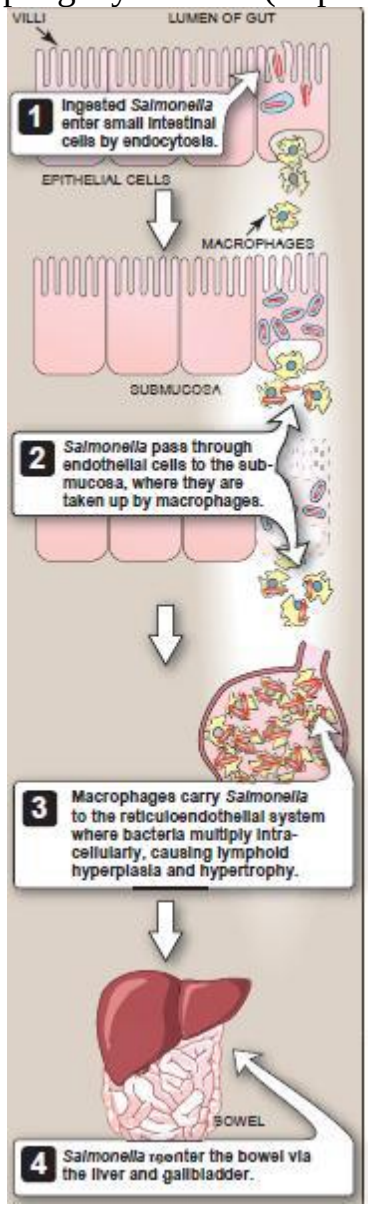
The salmonellas cause two distinct types of disease. One group of two species, Salmonella typhi and Salmonella paratyphi, is the cause of the enteric fevers, typhoid and paratyphoid. The other group, consisting of over 2000 serotypes of what is now considered to be one species, Salmonella enterica, causes gastroenteritis. These serovars were previously considered to be separate species and were named after the city or animal from which the organism was initially isolated.

Transmission of salmonellas is by the faecal oral route and often involves food and sometimes water. The enteric fever salmonellas are associated only with humans and human disease and remain important causes of waterborne disease worldwide, but nowadays very rarely in developed countries. The gastroenteritis salmonellas are widespread in animals and are often found in poultry, eggs and meat products. Food is the major vehicle of infection, but transmission via water does occur, even though the bacteria survive for only a few hours or days in surface water. Normal water treatment processes are adequate to remove the organism from drinking-water. The organisms are susceptible to chlorine disinfection.

The infectious dose for humans for the enteric fever salmonellas is about

10^2–10^3 organisms, whereas the infectious dose for humans for the gastroenteritis salmonellas is about 10^6–10^8 organisms, mainly because of their susceptibility to gastric acid.
The enteric fevers are systemic infections presenting with high fever (40–41 °C), headache, malaise and rigors. Diarrhea does not usually occur, and patients often experience constipation in early enteric fever.

Pathogenesis
Salmonella invade epithelial cells of the small intestine. Disease may remain localized or become systemic, sometimes with disseminated foci. The organisms are facultative, intracellular parasites that survive in phagocytic cells. (steps in pathogenesis as illustrated in the figure)



Clinical significance
Salmonella infection can cause both intestinal and extraintestinal diseases.

1. Gastroenteritis: This localized disease (also called salmonellosis) is caused primarily by serovars Enteriditis and Typhimurium.
In uncompromised patients, disease is generally self-limiting (48 to 72 hours).
2. Enteric or typhoid fever: This is a severe, life threatening systemic illness, characterized by fever and, frequently, abdominal symptoms. It is caused primarily by serovar Typhi.
About 30 percent of patients have a faint and evanescent (transient) maculopapular rash on the trunk (rose spots). The incubation period varies from 5 to 21 days. Untreated, mortality is approximately 15 percent.

Laboratory identification
In patients with diarrhea, Salmonella can typically be isolated from stools on MacConkey agar or selective media.
For patients with enteric fever, appropriate specimens include blood, bone marrow, urine, stool, and tissue from typical rose spots (if they are present).

SHIGELLA
Species of Shigella are the causative organisms of dysentery (shigellosis or bacillary dysentery) that occurs most commonly among **young children**, and are almost entirely human pathogens; no other animal species play a role in maintenance or spread of infection in the community. Occasionally, higher primates become infected by human-to-animal transmission. Shigellas are transmitted by the faecal–oral route and sometimes, because the infectious dose is low, around 10^2 organisms, by person-to-person spread. Patients excrete large numbers of organisms, between 10^5 and 10^8 per gram of faeces. Point source outbreaks associated with infected food handlers are reported from time to time. Occasionally, waterborne outbreaks occur, although the organism does not survive for more than a few hours or days in surface water, and normal water treatment processes are adequate to remove it from drinking-water.

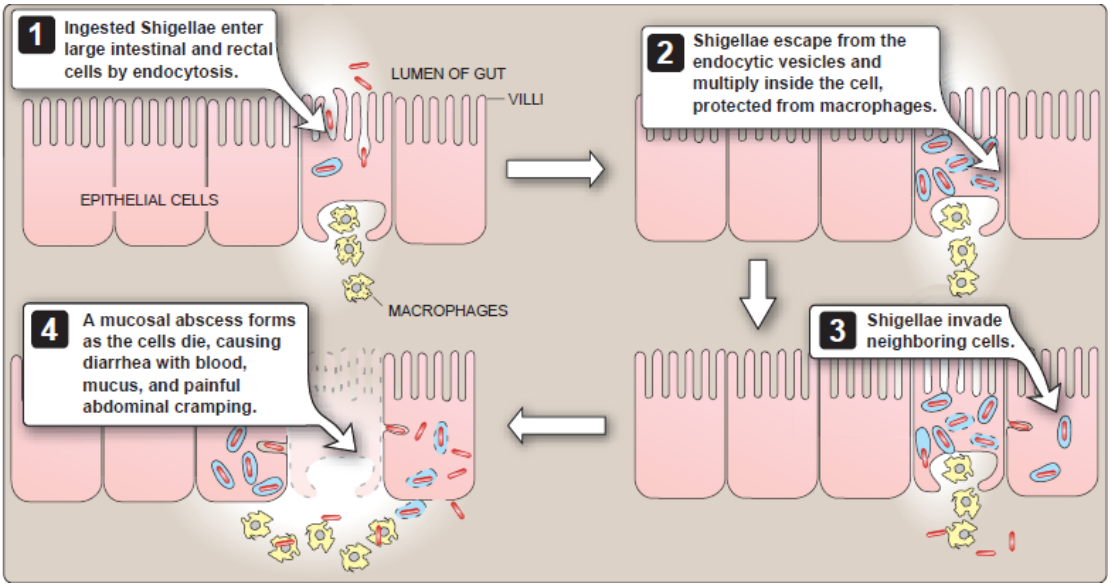
Shigellae are **nonmotile, unencapsulated**. The **40 serotypes** of Shigella are organized into four groups (A, B, C, and D) based on the serologic relatedness of their polysaccharide **O antigens**.

Pathogenesis & Pathology
incubation period (1–2 days)
Shigella infections are almost always **limited to the GIT**

Toxins
A. ENDOTOXIN: contributes to the irritation of the bowel wall.

B. *SHIGELLA DYSENTERIAE* EXOTOXIN

S dysenteriae type 1 (Shiga bacillus) produces a heat-labile exotoxin that affects both the gut and the central nervous system.



Laboratory identification

During acute illness, organisms can be cultured from stools using differential, selective **Hektoen** agar or other media specific for intestinal pathogens.

VIBRIO

The *Vibrio* genus is composed of over 30 species, of which the most important is *V. cholerae*, the cause of epidemic cholera, a predominantly waterborne infection. The species *V. cholerae* is subdivided into 140 O-serovars, of which the toxin-producing strains are O1 and O139. The epidemiological picture of cholera has changed and now has a wide distribution. The seventh pandemic that began in 1961 was caused by El Tor strains.

The O1 strain continues to occur in about 19.7% of patients. Cholera is a disease of humans, and approximately 5% of patients become carriers. The organism survives well in the environment, and viable but non-culturable organisms have been described. There is quite clearly potential for further epidemic spread. Other *Vibrio* species, particularly *Vibrio parahaemolyticus*, have been associated with diarrhoea, often through the consumption of raw, contaminated seafood. Vibrios are removed from raw waters by chlorination and normal water treatment processes.

Members of the genus Vibrio are short, curved, rod-shaped organisms. They are rapidly motile by means of a **single polar flagellum**.

O and H antigens are both present, but only O antigens are useful in distinguishing strains of vibrios that cause epidemics.
Vibrios are facultative anaerobes. The growth of many Vibrio strains either **requires** or is stimulated by **NaCl.**
Vibrio grow in synthetic media with glucose as carbon and energy source.
Vibrio tolerate alkaline and sensitive to acid.

Epidemiology
The infectious dose varies depending on the pH of the stomach, in healthy volunteers **10^8 bacteria** produce infection.
The **small intestine** is the primary site of infection. And *V. cholera* colonizes the epithelium without invasion or apparent damage.
cholera is characterized by **massive loss of fluid** and electrolytes from the body. After an incubation period ranging from **hours to a few days**, profuse watery diarrhea ("**rice-water**" stools) begins. Untreated, death from severe dehydration causing hypo-volemic shock may occur in hours to days, and the death rate may exceed 50 percent.

Virulance factor
Cholera toxin (CT) composed of AB subunits. Once inside the cell (A)subunit causes changes in the regulation of cell genes and the result flow of ions and water is reversed.

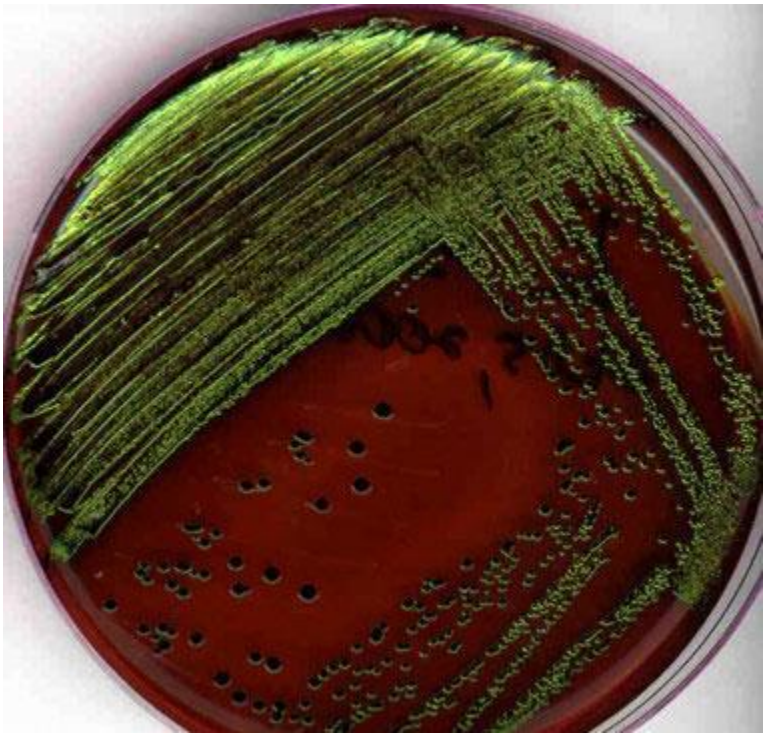
Laboratory identification
V. cholerae grows on standard media such as blood and MacConkey agars. Thiosulfate-citrate-bile salts–sucrose (TCBS) medium can enhance isolation and it required **transport media (Carry Blair media**).

Lab 4 Culture Media and Biochemical reactions

Culture Media:

Differential media:

a. Eosin Methylene blue (EMB) agar:
   - Contains the dyes eosin and methylene as well as the carbohydrates lactose-and sucrose. The dyes inhibite gram+ve bacteria and act as indicators of those bacteria capable of fermenting lactose (appear as dark-colored), lactose non-fermenter (translucent or colorless colonies).
   - EMB agar is particularly valuable in identifying Escherichia coli (green metallic sheen).



E.coli on EMB medium showing a green metallic sheen

b. MacConkey's Agar.

- Distinguishes between lactose fermenters (red or pink) and lactose nonfermenters (translucent or colorless).
- pH indicator, neutral red, which gives a red color under acid conditions.
- The growth of gram-positive bacteria is inhibited on MacConkey's agar because of the presence of bile salts and the dye crystal violet.

c. Deoxycholate Agar. very similar to MacConkey's agar (neutral red and distinguishes between lactose fermenters and lactose nonfermenters).

Selective media: Salmonella-Shigella (SS) agar

- Contains the pH indicator neutral red and the carbohydrate lactose.
- Contain salts (ferric citrate and sodium thiosulfate). The presence of these salts provides the medium with an indicator of hydrogen sulfide production. The colonies of bacteria producing hydrogen sulfide may have blackened centers, which is the result of the precipitation of ferric sulfide.

Biochemical Reactions

Catalase Test

Principle:

The test determines the ability of the organism to produce an enzyme called catalyze which breaks down hydrogen peroxide into oxygen and water. It is performed by the emulsification of a bacterial colony in hydrogen peroxide solution. If catalase is present, air bubbles will form due to the production of oxygen.

Procedure:

On a clean slide, put two drops of hydrogen peroxide. Using a loop, take a small amount of the bacterium and emulsify it on the slide. If bubbles form, the organism is catalase positive.

Oxidase test:

This test is used to differentiate those bacteria that produce the enzyme Oxidase from non-oxidase producer bacteria.

Method of the test:

1. Place a piece of filter paper in a clean Petri- dish.

2. Add 2 or 3 drops of oxidase reagent on the filter paper.

3. Using a wooden stick, smear a colony of the test organism across the reagent on the filter paper.

4. Observe the color change to deep blue-purple within 10 seconds.

Results:

- ✓ If the filter paper shows deep blue-purple within 10 seconds: **positive (ignore any blue-purple color that develops after 10 seconds).**
- ✓ If no color appears within 10 seconds: Negative.
- ✓ Pseudomonas, Neisseria, Vibrio and Brucella species: Oxidase positive.
- ✓ Escherichia coli and other **Enterobacteriaceae**: Oxidase **negative**.

IMViC test: **INDOLE TEST:** Principle:

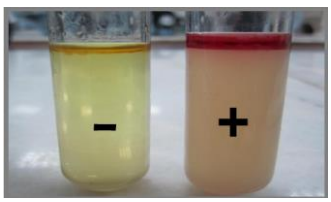
The test organism is cultured in a medium which contains tryptophan. This amino acid (Tryptophan) is broken down and indole is released. Indole production is detected by addition of Kovac's reagent which contains 4 (p)-dimethylaminobenzaldehyde which reacts with the indole to produce colored compound.

Method:

1. Prepare peptone water.
2. Inoculate the test colony to the tube containing peptone water.
3. Incubate overnight in 37 ˚C.
4. After incubation period, add drops of Kovac's reagent to the tube.
5. Shake gently and then examine for a red color (red ring) in the surface layer within 10 minutes.

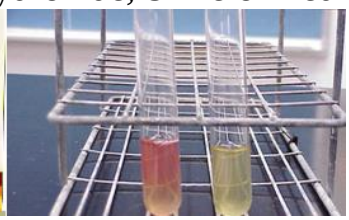
Results:

- ✓ After adding kavoc's reagent: red ring appears positive result for indole production (e.g. E.coli).
- ✓ If the reagent remain as yellow ring: it is negative and no indole produced.



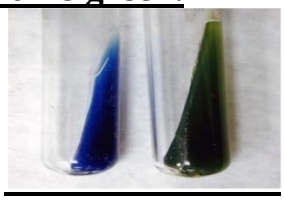
Methyl red-Voges proskauer test:

- **How to Perform Tests**: Inoculate 2 glucose broths with inoculating loop. After 48 hours of incubation, add a few drops of MR to one tube, and VP reagents to the other tube.
- **Properties they test for**: Both tests are used to differentiate species of the family Enterobacteriaceae.
  - MR—tests for acid end products from glucose fermentation.
  - VP—tests for acetoin production from glucose fermentation.
- **Media and Reagents Used**:
  - Glucose Broth
  - Methyl Red indicator for acid
  - Voges Proskauer reagents—A: 5% Alpha-Naphthol, & ethanol, B: Potassium Hydroxide, & Deionized Water.

Citrate test:

- **How to Perform Test:** Inoculate slant with inoculating loop.
- **Property of test:** This test is used to help differentiate species of the family *Enterobacteriaceae*. It is selective for bacteria that has the ability to consume citrate as its sole source of carbon and ammonium as sole nitrogen source.
- **Media and Reagents Used:** Simmon's Citrate Agar contains sodium citrate (carbon source), ammonium ion (nitrogen source), & pH indicator—bromthymol blue.
- **Reading Results:**
  - **positive result is blue** (meaning the bacteria metabolize citrate and produced an acid end product). **negative result the media remains green.**



TSI: triple sugar iron test

This agar contains three sugars are: lactose (10 parts), sucrose (10 parts), and glucose (1 part). The medium also contains a pH indicator (phenol red) and ferrous sulfate to detect hydrogen sulfide (H2S) production. TSI tube determine the following:

- ✓ can the organism ferment glucose, and if so, can it also ferment lactose and sucrose?
- ✓ Is gas produced as a byproduct of fermentation?
- ✓ Does the organism produce H2S?

TSI tube has a slant portion and butt (deep) portion, the uninoculated medium is red, meaning it is alkaline. The slant portion is aerobic and the butt portion is relatively unaerobic.

Method:

Inoculate the butt portion by stabbing the needle through the center of the medium until reaching the butt.

Inoculate the surface of the slant using zigzag motion.

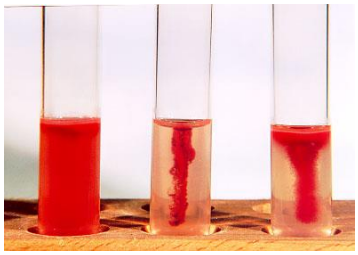
Inocubate the tube at 35c for 18-24hr.

Result:

Yellowing the butt indicates glucose fermentation. Yellowing the slant indicate that the organism ferment sucrose and/or lactose. Bubbles and /or cracks in the agar or separation of the agar indicate gas production. Blackening the medium indicate the H2S production.

Motility test:

- ➲ **How to Perform Test:** Stab motility media with inoculating needle.
- ➲ **Property of the test:** This test is done to help differentiate species of bacteria that are motile.
- ➲ **Media and Reagents Used:** Motility media contains tryptose, sodium chloride, agar, and a color indicator.
- ➲ **Reading Results:** If bacteria is motile, there will be growth going out away from the stab line, and test is positive. If bacteria is not motile, there will only be growth along the stab line. A colored indicator can be used to make the results easier to see.



UREASE TEST:

Testing for urease activity is important in differentiating enterobacteria that produce the enzyme urease from non-urease producers.

Principle:

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease-producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide.

Results:

- ✓ Urease positive organisms yield a bright pink or bright red color to the agar (e.g. Proteus species are strong urease producers).
- ✓ Yellow color indicates negative reaction and no urease enzyme in the test organism (e.g. E. coli).