GIS 4

MICROBIOLOGY

Done by: Hadeel Abdullah

Scientific Correction: Abdulrhman Al-Jashaam

Gramatical Correction: Abdulrhman Al-Jashaam

Doctor: Nader Al-Aridah

It's been a while hasn't it? Let's run-through some points;

The Enterobacteriaceae (*coliforms*) are a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. Some are part of the normal microbiota (e.g. *E. coli*) and **incidentally** cause disease, but others (e.g. *Salmonellae* and *Shigellae*) are regularly pathogenic for humans. They are **facultative anaerobes** or aerobes, possess a complex antigenic structure, and produce a variety of **toxins** and other **virulence factors**.

Salmonellae are often pathogenic for humans or animals when acquired by the **oral** route. They are transmitted from animals and animal products to humans, where they cause gastroenteritis, systemic infection, and enteric fever (infections might **overlap**).

- Most isolates are **motile** with peritrichous flagella, so they have **H** antigens (antigens located on flagella).
- They are gram-negative bacilli, so they possess **O** antigens.
- They have the virulent capsular **Vi** antigens.
- They grow readily on simple media, but they almost **never** ferment lactose or sucrose. They ferment glucose and mannose to form acid and sometimes gas (they ferment rather than **oxidize** glucose; they are oxidase **negative**).
    - Non-lactose fermenters = they give colorless colonies in Eosin methylene blue EMB or MacConkey agar plates.

- They usually produce H_2S (extra info: they reduce compounds that possess sulfur in order to obtain energy).
    - Recall that *Shigellae* **don't** produce H_2S (differential test).
- They **survive** freezing in water for long periods.
- They are **resistant** to certain chemicals (e.g. **brilliant green**, sodium tetrathionate, sodium deoxycholate) that **inhibit** other enteric bacteria; such compounds are therefore useful for **inclusion** in media to isolate *Salmonellae* from feces.

CLASSIFICATION

The classification of salmonellae is **complex and controversial**. Regardless of how, *Salmonella* is divided into **two species** each with multiple **subspecies and serotypes**. The two **species** are *Salmonella enterica* (causes human illness) and *Salmonella bongori* (associated with cold-blooded animals).

- Six serovars (serotypes) of salmonellae of medical importance:
    1. S. enterica subsp. **typhi** (causes typhoid fever).
    2. S. enterica subsp. **enteritidis** (causes gastroenteritis).
    3. S. enterica subsp. **typhimurium**.
    4. S. enterica subsp. **choleraesuis** (causes bacteremia and focal lesions).
    5. S. enterica subsp. **paratyphi**.
    6. S. enterica subsp. **dublin**.

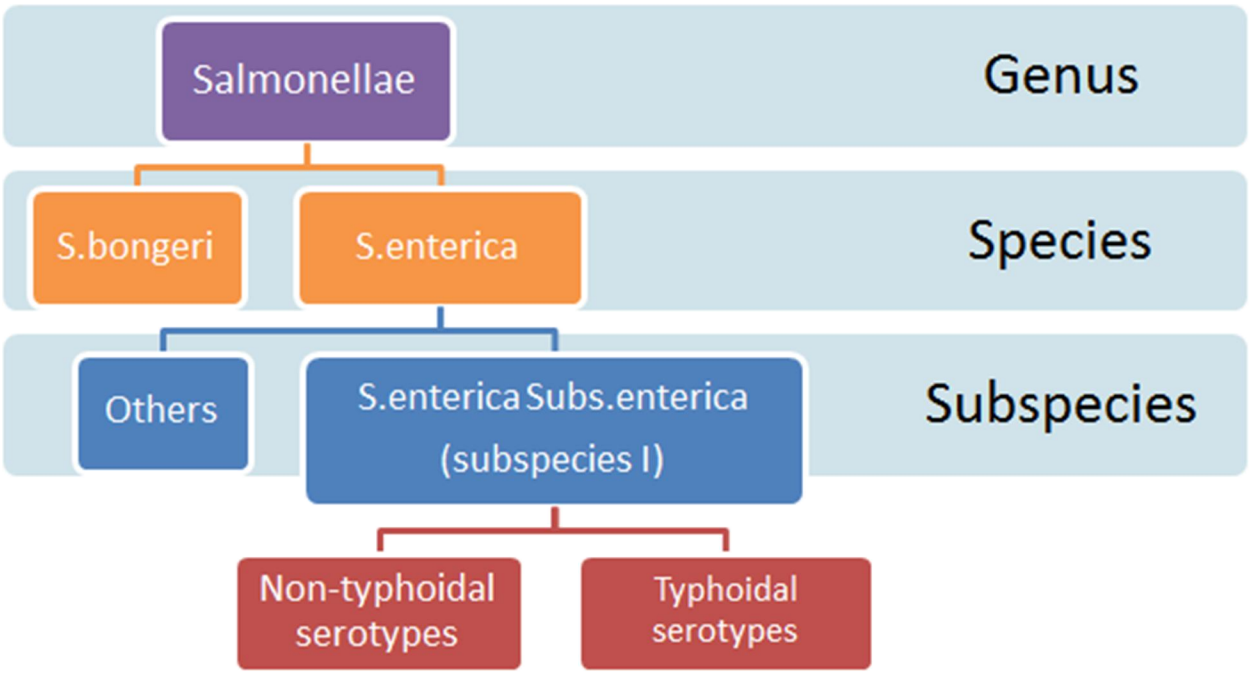
- *Salmonella enterica* serotypes can be divided into two main groups; typhoidal (cause typhoid fever that will be discussed) and non-typhoidal.

- **Four** typhoidal serotypes can be identified in the clinical laboratory by biochemical and serologic tests. These serotypes should be routinely identified because of their clinical significance. They are as follows:

1. Salmonella **paratyphi A** (serogroup **A**).
2. Salmonella **paratyphi B** (serogroup **B**).
3. Salmonella **paratyohi C** (serogroup **C**).
4. Salmonella **typhi**

Non-typhoidal *Salmonella* serotypes (NTS) primarily cause **gastroenteritis**, **bacteremia**, and **focal infections**. Many of these non-typhoidal infections are caused by $_1$ *S.enteritidis* and $_2$ *S. typhimurium* that cause **gastroenteritis**, $_3$*S.choleraesuis* that causes **bacteremia** and **focal infections**.

- **Enteritidis** and **Typhimurium** are the **two** most common serotypes reported in the developed world.
- I made this chart for more clarification, you won't find it in the slides.



Humans are the **only** reservoir for *Salmonella **typhi***, they can carry the bacteria in the **biliary tract** for very long times (chronic asymptomatic carriers, **not** normal flora), and transmit the bacteria to other persons (either **directly** $_1$ or **indirectly**$_2$ via food or water contamination by their feces). These individuals are a more important source of contamination than frank **clinical cases (symptomatic)** that are promptly isolated, such as when carriers working as food handlers are "shedding" organisms.

However, the vast majority of *Salmonellae* (other than *S.typhi*) are chiefly **pathogenic** in **<u>animals</u>** that constitute the **reservoir** for human infection as they carry the bacteria in their tissues, excreta, or eggs (e.g. cattle, rodents, and fowl).

- Recall:

Shigella, Salmonella, and *Yersinia* are **not** normally part of the human microbiome and are **always** considered pathogenic if found there.

THE "ENTERIC FEVERS" (TYPHOID FEVER)

Typhoid is a **severe** systemic disease caused by *S. typhi, S. paratyphi A, B,* and *C.* It is the **<u>most</u> severe** presentation of salmonellosis (a major cause of morbidity and **mortality** worldwide).

- The characteristic feature of typhoid is that it is a **month-long fever**.
- As mentioned above, *Salmonella typhi* can colonize the **gallbladder** and persist in an **asymptomatic carrier** state that is frequently associated with the presence of **gallstones.**

- Therefore, it is strictly a human disease and must be traced back to a human **reservoir**.

- Its incidence differs significantly developing vs. developed counties 0.2-4 cases to up to 500 /10^5 population.

PATHOGENESIS

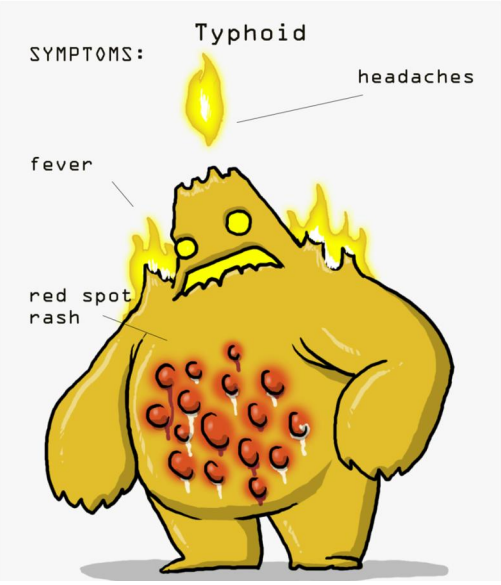
The organisms **almost** always enter via the **oral** route - vertical transmission (trans- placental) is **possible-** , **usually** with contaminated food or drink, and among the host factors that contribute to resistance to salmonella infection are **gastric acidity$_1$**, **normal intestinal microbiota$_2$**, and local intestinal **immunity$_3$** (in other words, stomach acidity and normal intestinal microbiota are important determinants of **susceptibility**; acidity and intestinal bacteria can kill the *Salmonella* before it has the opportunity to invade cells and replicate).Therefore, the mean infective dose to produce clinical or subclinical infection in humans is **high** (10^5 to 10^8), i.e. low doses are **not** sufficient to cause the disease. Thank god.

- After ingestion, infection with *Salmonellae* is characterized by attachment of the bacteria to cells lining the intestinal lumen. *Salmonellae* selectively attach to specialized epithelial cells (M or microfold cells) of the Peyer's patches (just like *Shigellae*).
- The bacteria are then **internalized** by endocytosis (within vacuoles or phagosomes) and transported to the lamina propria, where they are **released** into the lymphatic circulation.
- Once the bacteria reach intestinal lymph nodes, they multiply in **mononuclear cells** to **mesenteric** lymph nodes to **blood** through thoracic duct (transient bacteremia).

- Along their journey through the mesenteric lymph nodes to the **thoracic duct**, they invade the **reticuloendothelial tissues** of the liver, spleen, and bone marrow.
- **Circulating** endotoxin cause prolonged fever (endotoxin is a component of the exterior cell wall of gram-negative bacteria).
- Inflamed mucosa and lymphatics, necrosis and sloughing of overlaying epithelium.
- Producing ulcer that may bleed, however, these ulcers heal without scarring.
- Cell mediated immunity is important (it's an intracellular pathogen).

Extra: recall that *Shigellae* **escape** from their vacuoles and are **not** transported within phagosomes into the lamina propria to be **released** into the circulation. Instead, they become free and invade adjacent epithelial cells.

CLINICAL MANIFESTATIONS



- The incubation period ranges from **7-14** days.
- Onset is insidious.
- During the **1st** week (the week after the incubation period): Fever, malaise, anorexia, myalgia, headache, abdominal pain, diarrhea (early), and constipation (later) occur. The increase in temperature in a **stepwise** fashion become unremitting and high (i.e. the fever rises **gradually** to a high **plateau** (becomes constant)).

- In the **2nd** week:
  High fever, fatigue, cough, epistaxis, abdominal symptoms more sever and rose spots are seen (skin rash or macules on chest and back).
- In the **3rd- 4th** week:
  If no complications arise, symptoms and signs **gradually resolve** on their own.
- In the pre-antibiotic era, the chief complications of enteric fever were intestinal hemorrhage and perforation, and the **mortality** rate was **10–15%**. Now, after antibiotics, mortality decreases ↘ to **less** than **1%**.
- The primary diagnostic method is blood culturing.

ENTEROCOLITIS

- This is the **most common manifestation** (**CLINICAL**) of *Salmonella* infection (typhoid fever is the most **serious** manifestation).
- In the United States, *S.typhimurium* and *S. enteritidis* are prominent, but enterocolitis can be caused **by any** of the more than 1400 group I serotypes (*subs.enterica*) of *Salmonellae*.
- As we said, the reservoirs are infected **animals and their products** specifically poultry, eggs, and dairy.

CLINICAL MANIFESTATIONS

- **Eight** to **48** hours after ingestion of salmonellae, there is nausea, headache, vomiting, and non-bloody **diarrhea**, with few leukocytes in the stools. **Low-grade** fever is **common**, but the episode usually resolves in **2–3 days**.

- Inflammatory lesions of the small and large intestine are present. However, bacteremia is **rare** (2–4%) except in **immune-deficient persons**.
    - Intestinal lesions > positive.
    - Bacteremia > negative.

- Blood culture results are **usually negative (no bacteremia)**, but stool culture results are **positive** for *Salmonellae* and may remain **positive** for several **weeks** after clinical recovery.
    - Blood > negative.
    - Stool > positive.

BACTERAEMIA WITH FOCAL LESIONS

It is associated commonly with *S. Choleraesuis* but you have to know that **all *Salmonellae* can cause bacteremia**.

- After oral infection, there is **early** invasion of the bloodstream with possible focal lesions in lungs, bones, meninges, and so on… but intestinal manifestations are often **absent**.
- It is no wonder that blood cultures are **positive** (bacteremia).
- Bacteremia is associated with **local suppurative** infections seeding from an organ, usually the bone causing osteomyelitis or the joints in the form of arthritis in more than 10% of the patients.
- Bacteremia depends on the serotype and the patients' health state.
    - Sickle cell anemia patients (sicklers) are more likely to develop *Salmonellae* bacteremia.
- The primary diagnostic method is **blood culturing.**

DIAGNOSTIC LABORATORY TESTS (FOR ALL SALMONELLA INFECTIONS) (I - III)

I) SPECIMENS:

- Blood for culture must be taken repeatedly.
In **enteric fevers** and **septicemias (bacteremia)**, blood culture results are often **positive** in the first week of the disease. Bone marrow cultures may be useful.
-Urine culture results may be positive after the second week.
- Stool specimens also must be taken repeatedly. In **enteric fevers,** the stools yield **positive** results from the second or third week on, while in **enterocolitis**, the stools yield **positive** results during the **first** week (early).
- A **positive** culture of duodenal drainage establishes the presence of salmonellae in the **biliary tract** in **carriers (asymptomatic).**

II) BACTERIOLOGIC METHODS FOR ISOLATION OF SALMONELLAE

1. Enrichment cultures: the specimen (usually stool) is put into "selenite F" or "tetrathionate broth", both of **which inhibit replication of normal intestinal bacteria$_1$** and **permit multiplication of salmonellae$_2$.**
2. Differential and Selective medium cultures:
    A) Differential medium culture: **EMB, MacConkey**, or deoxycholate medium permits rapid detection of lactose non-fermenters (not only salmonellae). Gram-positive organisms are somewhat inhibited. Bismuth sulfite medium permits **rapid detection** of *Salmonellae*, which form black colonies because of H_2S production.

**** EMB and MacConkey agar plates promote the growth of Enterobacteriaceae family and differentiate between them in lactose fermentation.**

B) Selective medium culture:
The specimen is plated on salmonella-shigella (SS) agar, Hektoen enteric agar, xylose-lysine decarboxylase (XLD) agar.

3. Final identification: Suspect colonies from solid media are identified by **biochemical** reaction (e.g. H_2S production) patterns and slide agglutination (**serological**) tests with specific sera.

III) SEROLOGIC METHODS

1. Agglutination test: in this test, **known** sera (contain antibodies specific for *Salmonellae* antigens) and **unknown** (**suspected** to carry *Salmonellae* colonies) culture are mixed on a slide.
    - **Clumping (positive)**, can be observed within a few minutes. This test is particularly useful for rapid preliminary identification of cultures. There are commercial kits available to agglutinate and sero-group *Salmonellae* by their O antigens: A, B, C1, C2, D, and E.
2. Tube dilution agglutination test (Widal test):
   In this test we look for *Salmonellae* antibodies in the patient's serum using antigens that are **specific** for *Salmonellae* (from the laboratory).
    - Serum agglutinins (antibodies) rise sharply during the second and third weeks of *S.typhi* infection (in the patient's serum). The Widal test detects these antibodies against the O and H antigens.

- Serial dilutions of **unknown sera** (here, the sera are **unknown**; because we don't know whether there are *Salmonella*-specific antibodies or not) are tested against antigens from **representative** *Salmonellae*.
    - False-positive and false-negative results occur.
- The interpretive criteria when single serum specimens are tested **vary**, but a titer against the **O** antigen of **greater than 1:320** and against the **H antigen** of greater than **1:640** is considered **positive**.
    - O antigen > greater than one over 320 titer
    - H antigen > greater than one over 640 titer
- Results of serologic tests for *Salmonella* infection **cannot** be relied upon to establish a definitive diagnosis because of the cross reactivity between antigens that may occur due to **immunization** (against typhoid for example) or previous intestinal infections other than *Salmonella* infections.

**High titer of antibody to the Vi antigen occurs in some carriers (not mentioned).

**At least two serum specimens, obtained at intervals of 7–10 days, are needed to prove a rise in antibody titer (not mentioned).

**Alternatives to the Widal test include rapid colorimetric and EIA methods (not mentioned).

IMMUNITY

- Infections with S serotype *Typhi* or *Paratyphi* usually confer a certain degree of immunity. However**, reinfection may occur** but is often milder than the first infection.

- Circulating antibodies to O and Vi are related to resistance to infection and disease. However, **relapses may occur** in 2–3 weeks after recovery despite antibodies.
- Secretory **IgA** antibodies may prevent attachment of *Salmonellae* to intestinal epithelium (IgA antibodies are the cornerstone of immunity in *Salmonella* infections)
- As we mentioned before, persons with **S/S hemoglobin** (sickle cell disease) are exceedingly susceptible to *Salmonella* infections, particularly **osteomyelitis**. Persons with A/S hemoglobin (sickle cell trait) may be more susceptible than normal individuals (those with A/A hemoglobin).

Extra info: hemoglobin S (Hgb S) is an abnormal type of hemoglobin that you can inherit from your parents. While hemoglobin A (Hgb A) is the normal type of hemoglobin in the red blood cells.

TREATMENT

- Although enteric fevers and bacteremia with focal lesions **require antimicrobial** treatment (usually ciprofloxacin or cephalosporin), the vast majority of cases of **enterocolitis** do **not**. Antimicrobial treatment of *Salmonella* enteritis in neonates is important.
  - In enterocolitis, clinical symptoms and excretion of the *Salmonellae* may be prolonged by antimicrobial therapy. In severe diarrhea, replacement of fluids and electrolytes is essential.

- Antimicrobial therapy of invasive *Salmonella* infections is with ampicillin, fluoroquinolones, trimethoprim–sulfamethoxazole, or a third-generation cephalosporin
- Multiple drug resistance transmitted genetically by plasmids among enteric bacteria is a problem in *Salmonella* infections. Susceptibility testing is an important adjunct to selecting a proper antibiotic.
- In most carriers, the organisms persist in the **gallbladder** (particularly if gallstones are present) and in the biliary tract.
- Some **chronic carriers** have been cured by **ampicillin** alone, but in most cases **cholecystectomy** (removal of gallbladder) must be combined with drug treatment hence it is the most common site of colonization.

PREVENTION AND CONTROL

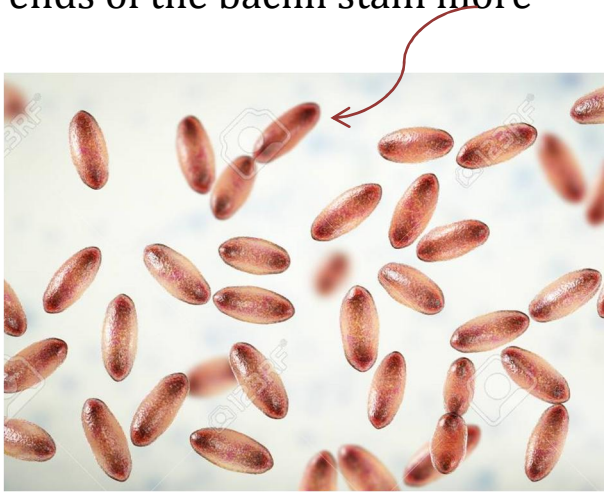
- Three percent of survivors of typhoid become healthy permanent carriers, harboring the organisms in the **gallbladder**, biliary tract, or –rarely- the intestine or urinary tract.
- **Sanitary measures** must be taken to prevent contamination of food and water by rodents or other animals that excrete salmonellae.
- Infected poultry, meats, and eggs must be thoroughly **cooked**.
- Carriers must not be allowed to work as **food handlers** and should observe strict hygienic precautions.
- Two typhoid ***symptomatic form*** vaccines are currently available: an oral live, attenuated vaccine and a Vi capsular polysaccharide vaccine for intramuscular use. Vaccination is recommended for travelers to endemic regions, especially if

the traveler visits rural areas or small villages where food choices are limited, efficacy of 50–80% (not that much; it gives **short-lived immunity**).

Salmonellae √

Yersinia comprises gram-negative bacteria of the family Enterobacteriaceae (gamma proteobacteria ←the class name)

- *Yersiniae* are short, pleomorphic (cocco-bacilli) rods that can exhibit bipolar staining (i.e. the ends of the bacilli stain more intensely than the middle).



- Most have animals as their natural hosts, but they can produce serious disease in humans –occasionally-.
- They grow **best** (optimally) at 25°C and are motile at 25°C but **non**-motile at **37°C (body temperature).**
- ***Y. pestis*** –the cause of plague (black death)- is transmitted to humans usually through the bite of an infected flea, although inhalation is another potential route.
- **Yersiniosis** is a **zoonotic** infection with an enteropathogenic Yersinia species, usually ***Y. enterocolitica*** or ***Y. pseudotuberculosis*.**
- *Y. enterocolitica* exists in more than 70 serotypes; most isolates from human disease belong to serotypes O:3, O:5, O:8, and O:9.

- *Y. enterocolitica* has been associated with transfusion related infections caused by contaminated red blood cells. This is a consequence of the ability of the organism, transmitted by an asymptomatic donor, to multiply at **refrigeration** temperatures.

**Y. enterocolitica is found worldwide and has been isolated from a wide variety of wild and domestic animals and environmental samples, including samples of food and water (not mentioned).

**Most clinical infections are associated with sero-groups O:3, O:9, and O:5,27, with a declining number of O:8 infections (not mentioned).

** Consumption or preparation of raw meat, products milk (pasteurized, unpasteurized, and chocolate-flavored) and various foods contaminated with spring water products are linked with infection (not mentioned).

** Y. pseudotuberculosis is less frequently reported as a cause of human disease than Y. enterocolitica (not mentioned).

PATHOGENESIS

- Transmission to humans probably occurs by contamination of food, drink, or fomites (transmitted orally).
- Initial replication in the small intestine is followed by invasion of Peyer ' s patches of the distal ileum via **M cells**, with onward spread to **mesenteric lymph nodes** (remember *Salmonellae*?).
- The liver and spleen can also be involved after oral infection.
- The characteristic histologic appearance of entero-pathogenic *Yersiniae* after invasion of host tissues is as extracellular micro-abscesses surrounded by an epithelioid granulomatous lesion.

- *Y. enterocolitica* can produce a **heat-stable enterotoxin$_1$**, but the role of this toxin in diarrhea associated with infection is not well defined.
- All *Yersiniae* possess lipopolysaccharides (gram-negative) that have **endotoxic activity $_2$**when released.
- They have **type III secretion systems $_3$** that consist of a membrane-spanning complex that allows the bacteria to inject proteins directly into cytoplasm of the host cells.
- The pathogenic *Yersiniae* have **a pathogenicity island (PAI)$_4$** that encodes for an iron scavenging siderophore.

CLINICAL MANIFESTATIONS

- Y. *enterocolitica* is more closely associated with terminal ileitis and Y. *pseudotuberculosis* with **mesenteric adenitis**, **but both organisms may cause mesenteric adenitis** and symptoms of **abdominal pain, fever, and tenderness** that result in **pseudo-appendicitis**, with the surgical removal of a normal appendix. (i.e. *Y. enterocolitica* and *Y. pseudotuberculosis* are **possible** infectious **agents** in pseudo-**appendicitis**).
- ***Yersinia* gastroenteritis** is the most common reported presentation in infection with pathogenic *Y. enterocolitica*, especially in **children** under the age of 4, who form the single largest group in most case series. It is characterized by a self-limiting diarrhea, fever, and abdominal pain.
- **Post-infective** phenomena of **reactive arthritis** might be developing within 2–4 weeks of a preceding infection.

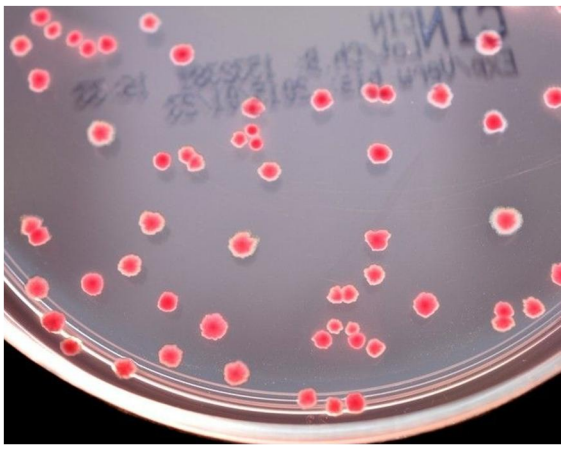
- Blood may be detected in diarrheal stool.
- Older children (>4 y/o) and adults are more likely than younger children to present with **abdominal pain**, which can be localized to the right iliac fossa (appendix site), a situation that often leads to laparotomy for **presumed** appendicitis (pseudo-appendicitis).

- Gastrointestinal **complications** include granulomatous appendicitis, a chronic inflammatory condition affecting the **appendix**.

LABORATORY DIAGNOSIS

1. Specimens:

- Specimens may be **stool$_1$**, **blood$_2$**, or material obtained at surgical **exploration$_3$**.



2. Culture:

- The number of *Yersiniae* in stool may be small and can be increased by **"cold enrichment" (why cold? because it grows optimally at 25 C ^{0})** where many fecal organisms do not survive, but *Y. enterocolitica* multiplies.
- Then, subcultures made at intervals on MacConkey agar may yield *Yersiniae*. Alternatively, most clinical laboratories use a *Yersinia* selective agar such as **cefsulodin-Irgasan-novobiocin (CIN)** agar incubated at **room temperature** for several days.
- Y.enterocolitica colonies have a bull's eye appearance with a red center on CIN agar.

Extra info: Subcultures are made by transferring some or all cells from a previous culture to fresh growth medium, in order to prolong the life or expand the number of cells and microorganisms in the culture.

3. Serology

- Serum specimens taken 2 or more weeks apart, a rise in agglutinating antibodies can be shown; however, **cross-reactions** between *Yersiniae* and other organisms (*Vibrios*, *Salmonellae*, and *Brucellae*) may **confuse** the results. Therefore, results are not reliable.

TREATMENT

- Most cases of diarrhea caused by entero-pathogenic *Yersinia* are self-limiting. Data from clinical trials **do not support** antimicrobial treatment for adults or children with ***Y. enterocolitica*** diarrhea.
    - Side note: *Y. enterocolitica* and *Y. pseudotuberculosis* are the two entero-pathogenic *Yersiniae*.
- *Y. enterocolitica* is typically resistant to **ampicillin$_1$** and to **first-generation cephalosporin$_2$**. On the other hand, *Y. pseudotuberculosis* has shown susceptibility to **ampicillin$_1$**, **cephalosporin$_2$**, and **aminoglycosides$_3$**.

Systemic infections with bacteremia or focal infections **outside the **gastrointestinal** tract generally **require antimicrobial** therapy (not mentioned).

** Fluoroquinolone therapy is effective for bacteremia in adults, such as ciprofloxacin, a third-generation cephalosporin is an alternative (not mentioned).

PREVENTION AND CONTROL

1. Safe handling and processing of food.
2. No vaccine is effective in preventing intestinal colonization of food animals by entero-pathogenic *Yersinia*.
3. Consumption of food made from raw meat should be **discouraged** at present because it is not possible to eliminate contamination with the entero-pathogenic *Yersinia* strains found worldwide.

Yersinia √