Chapter 18

Enteric Gram negative bacilli (1)

There are different classifications. A useful classification (though there is some overlap) is as follows:

1. **Pathogens within and outside the enteric tract**
2. **Pathogens primarily within the enteric tract**
3. **Pathogens outside the enteric tract**

Salmonella, Shigella and ***Campylobacter*** are frequent pathogens in GIT; ***Escherichia, Vibrio,*** and ***Yersinia*** have lesser frequency as pathogens.

UTI are primarily caused by ***E.coli***.

Family *Enterobacteriaceae*

Enterobacteriaceae is a large family of **Gram negative rods** found primarily in the **colon of humans and other animals**, many as normal flora.

Endotoxin is an essential component; many produce **exotoxins (enterotoxins)** which **activate adenylate cyclase** within the cells of small intestine and produce **diarrhea**.

Features **common to all members** are their anatomical location and following **four** metabolic processes:

1. All are **facultative anaerobes**
2. All **ferment glucose;** fermentation of other sugars vary
3. All are **oxidase negative --** none has cytochrome oxidase
4. All **reduce nitrates to nitrites** to gain energy**.**

Three types of antigens are important for identification and epidemiological purposes:

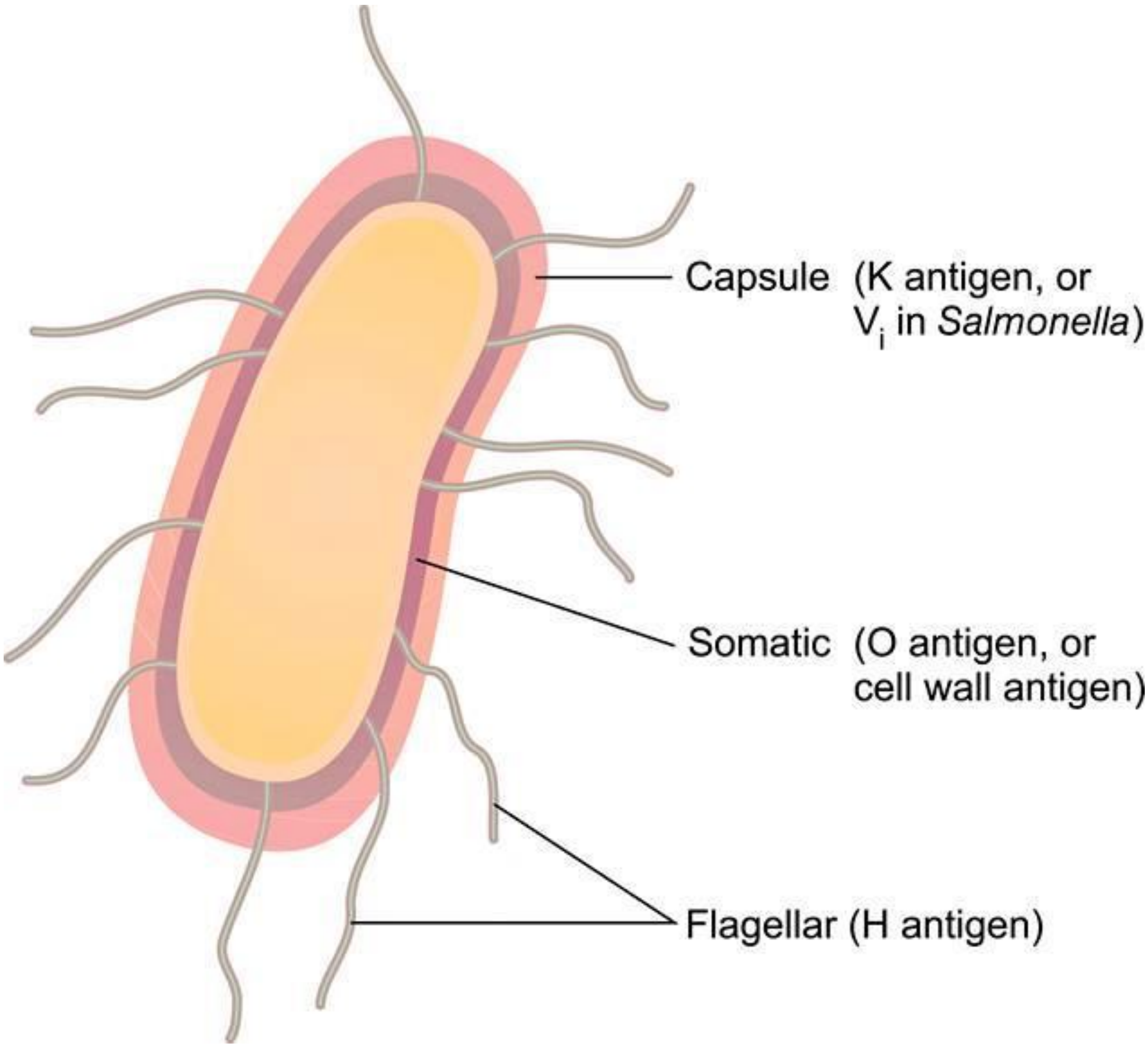
1. **Cell wall, somatic, or O antigen**. It is the outer polysaccharide portion of the lipopolysaccharide. It is formed of repeating units of 3-4 sugars.

   Their number is more than 2000 for ***Salmonella*** and 150 for ***E. coli***.

2. **Flagellar or H antigen** which are present only in **motile** bacteria.

   Some H antigens of ***Salmonella*** are unusual in being **biphasic**. This property helps in evasion of immune response.

3. **Capsular or K antigen** which is prominent in heavily encapsulated organisms such as ***Klebsiella***. K antigen is identified by **Quelleng reaction** and is useful in serotyping of ***S*. Typhi** and ***E.coli***.

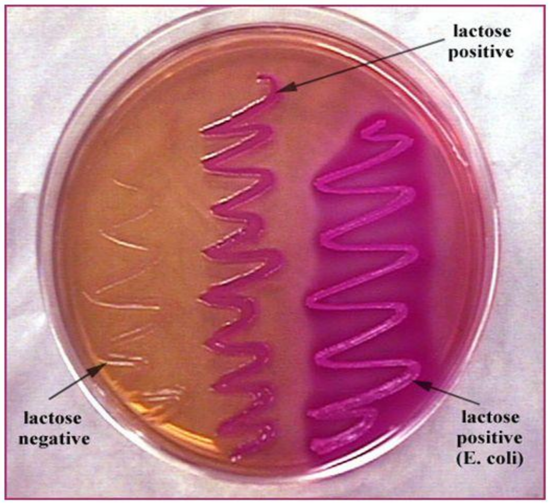

Capsule (K antigen, or
V_i in *Salmonella*)
Somatic (O antigen, or
cell wall antigen)
Flagellar (H antigen)

-- **Lactose fermentation** is a useful character for further differentiation. Selective differential media such as **MacConkey agar** or **EMB** agar are used.

-- **Non lactose fermenters** (e.g. ***Salmonella*** and ***Shigella***) form **colorless** colonies and **lactose fermenters** form colored usually **opaque** colonies.

-- Unwanted organisms are not able to grow due to **selective effect**.

MacConkey Agar



EMB agar

-- Additional use of **Triple Sugar Iron (TSI) agar** and **urea agar** helps in identifying the genus.

-- **Extensive biochemical testing** is required for identification at species level.

TSI agar

Urea agar

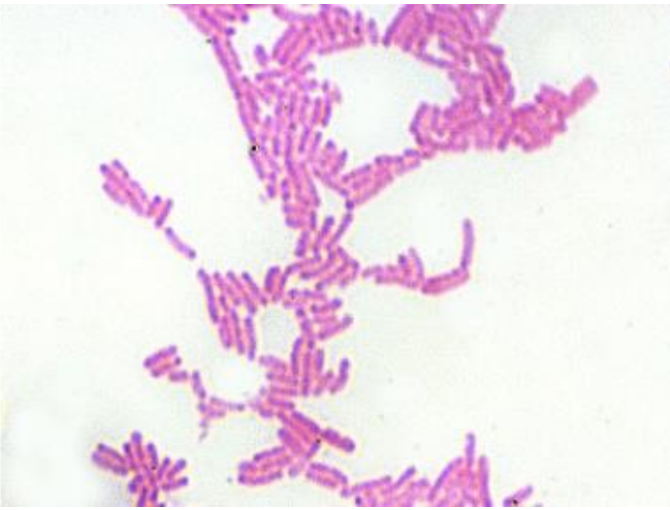
Pathogens within and outside the enteric tract

Escherichia

E.coli are one of the most common causes of **UTI, Gram negative sepsis, neonatal meningitis** and **traveler's diarrhea**.

Important properties

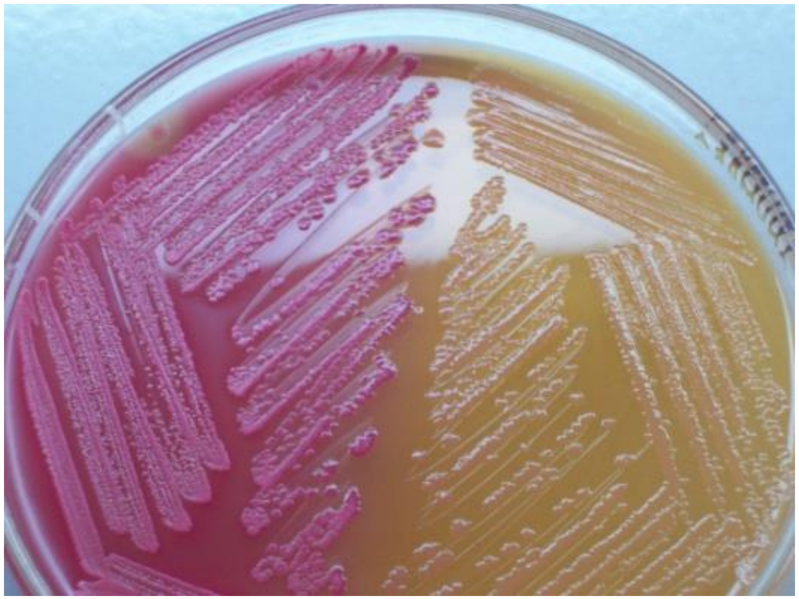
-- ***E.coli*** are **straight** Gram negative rods in contrast to curved rods of genera ***Vibrio, Campylobacter***, and ***Helicobacter***.

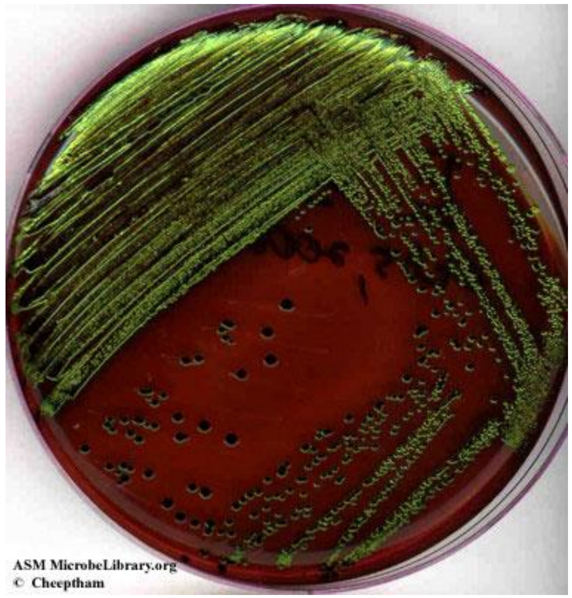


-- ***E.coli*** are the most abundant **facultative anaerobes** in colon and feces.

-- They **ferment lactose** which differentiates them from ***Salmonella*** and ***Shigella***.

-- O, H, and K antigens are used for further subtyping, and there are more than **1000** subtypes.





Pathogenesis

-- *E.coli* causing **UTI** originate from **patient's colon**.

-- Source of *E. coli* causing **neonatal sepsis** is **mother's birth canal.**

-- The strains which cause **traveler's diarrhea** originate from **food**, e.g. ***E.coli* 157**.

-- **Virulence factors** include **pili, capsule, endotoxin** and **three exotoxins** (**enterotoxins**)

-- **Two** exotoxins cause **watery** and **one bloody** diarrhea and **hemolytic-uremic syndrome.**

Intestinal tract infection

Once attached to intestinal tract by pili, the bacteria synthesize exotoxins which act on cells of **jejunum** and **ileum** to cause diarrhea. Colon cells are not affected.

One or both of following toxins may be produced:

1. **Heat-labile Toxin (LT):** It stimulates **adenylase cyclase** by catalyzing addition of adenosine diphosphate ribose to **G protein** (ADP-ribosylation).

   Iron transporters **export ions** which causes an **outpouring** of fluid, potassium, and chloride from the enterocytes into the lumen of gut resulting in watery diarrhea. Cholera toxin has similar action.

2. **Low MW heat stable toxin (ST)** which stimulates **guanylate cyclase**.

   These strains do not cause inflammation or invade intestinal mucosa.

-- Some others strains are **enteropathic (enteroinvasive)** and cause disease by **invasion of the epithelium of large intestine** causing **bloody diarrhea (dysentery)** accompanied by inflammatory cells (neutrophils) in the stool.

-- **Bloody diarrhea** may also be caused by certain **enterohemorrhagic strains** due to an exotoxin called **Shiga toxin**.

It acts by **removing an adenine from 28S ribosomal RNA**, thereby **stopping protein synthesis**. This toxin is encoded by temperate (lysogenic) bacteriophages.

-- The outbreaks are by **eating undercooked** food especially hamburger.

-- **Direct contact with animals** may also be a cause.

-- There can be a life threatening complication called **hemolytic-uremic syndrome (HUS)** characterized by hemolytic anemia, thrombocytopenia, and acute renal failure.

It occurs when Shiga toxin **enters blood stream**.

Antimicrobial treatment, e.g., ciprofloxacin increase the risk of HUS by increasing the amount of Shiga toxin released by dying bacteria.

Systemic infection

-- **Capsule** and **endotoxin** are main factors.

-- **Capsule interferes with phagocytosis** thereby helping in colonization; **K1 antigen** is associated with **neonatal meningitis**.

-- **Endotoxin** causes fever, hypotension and disseminated intravascular coagulation.

Urinary tract infections

-- Certain O serotypes of ***E.coli*** preferentially cause **UTI**.

-- These **uropathic** strains have **adhesion proteins in pili** which are specific for certain receptors on urinary tract epithelium.

-- The **motility** of ***E.coli*** may aid its ability to ascend the urethra into the bladder and ascend the ureter into kidney.

Clinical findings

Within intestinal tract

-- Diarrhea caused by **enterotoxigenic *E.coli*** is usually **watery, nonbloody**, self-limited, and of short duration (1-3 days).

It is frequently **associated with travelers**.

-- **EHEC (enterohemorrhagic) *E.coli*** cause **bloody diarrhea**, abdominal cramping and fever.

-- **STEC** cause **bloody diarrhea** and **HUS**. HUS in children is usually caused by **unnecessary use of antimicrobials.**

Outside intestinal tract

-- *E.coli* is leading cause of **community acquired UTI**.

-- These infections occur **primarily in women** because of three features that facilitate ascending infection into the bladder namely short urethra, proximity of urethra to anus and colonization of vagina by members of fecal flora.

-- It is also the **most common cause of nosocomial UTI** which has equal gender distribution and is usually caused by catheters.

-- Infections may be limited to **bladder (cystitis)** or move up to **kidney (pyelonephritis).**

-- Common symptoms of **cystitis** are pain (dysuria) and frequency of urination, and those of **pyelonephritis** are fever, chills and flank pains.

-- ***E.coli***, **along with Group B streptococci** is the major cause of **neonatal meningitis** and **sepsis**.

Transmission is from mother where these bacteria reside in vagina.

-- ***E.coli*** is also the main cause of **hospital acquired sepsis**.

-- ***E.coli*** may also cause **peritonitis** in combination with ***Bacteroides*** and ***Fusobacterium***.

Laboratory Diagnosis

Suspected specimens are cultivated on **blood agar** or **MacConkey/EMB** agar.

On **MacConkey agar** they form **rose pink colonies** whereas on **EMB agar** they have a **characteristic green sheen**.

It can be differentiated from other lactose fermenters by following characters:

1. **Production of indole from tryptophan**
2. **Decarboxylation of lysine**
3. **Use of acetate as only source of carbon**
4. **Motility**

***E.coli* 0157:H7** which produces Shiga toxin **does not ferment sorbitol** where as others do.

Treatment

-- It depends on **site of infection** and **drug resistance pattern** of specific isolate.

-- **Uncomplicated lower UTI** can be treated in 1-3 days with oral **trimethoprim-sulfamethoxazole or ampicillin**, but ***E.coli* causing sepsis** may need third generation **cephalsporins** and **aminoglycosides**.

-- For **neonatal meningitis**, a combination of **ampicillin and cefotaxime** is usually given.

-- In ***E.coli*** **diarrhea**, no antibiotic is required, however, trimethoprim-sulfamethoxazole and loperamide (Imodium) can shorten the duration. **Rehydration is more important**.

Prevention

-- There is **no specific prevention** such as immunization.

-- Incidence of UTI can be lessened by judicious use and **prompt removal of catheters**, and in **recurrent** cases with prolonged prophylaxis with specific antibiotics, e.g., **nitrofurantoin**.

-- **Cranberry juice** can be helpful because its tannins inhibit binding of pili.

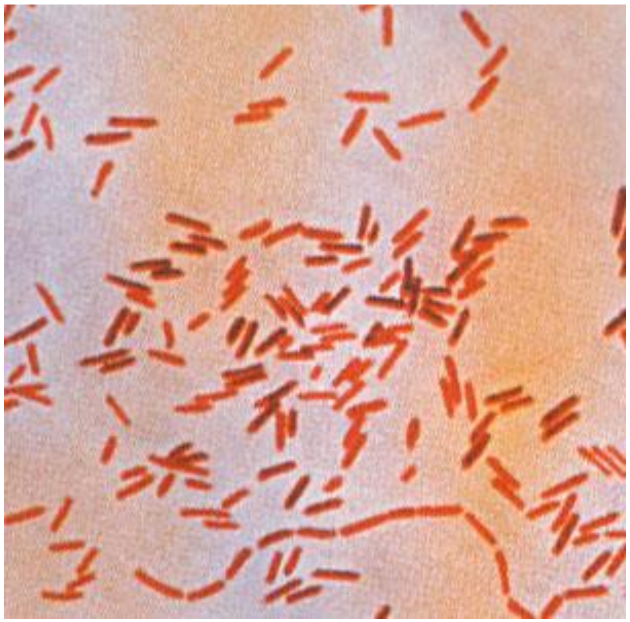
-- Traveler’s diarrhea can be prevented by **prophylactic antimicrobial use**.

Salmonella

Salmonella species cause **enterocolitis**, **enteric fevers**, and **septicemia** with **metastatic infections** such as **osteomyelitis**.

Important properties

Salmonellae are Gram negative rods that **do not ferment lactose** but do produce H_2S.



-- **Somatic (O), flagellar (H)** and **capsular (Vi)** are used for **taxonomic** and **epidemiological** purposes.

-- On basis of **O antigen**, Salmonellae are divided into **groups A-I**.

-- There are **two H types, phase 1 and 2** which are switched according to transcription from mRNA.

-- **Vi antigen** is antiphagocytic.

-- Most of pathogenic Salmonellae are serotypes of one species ***Salmonella enterica***. Other species is ***Salmonella bongori.***

-- Latest nomenclature writes **typhoid bacilli** as ***Salmonella enterica*** serovar **Typhi** or ***Salmonella* Typhi** .

-- Old nomenclature would simply write it as ***Salmonella typhi***.

-- Clinically, the division is into **typhoidal** (e.g., ***S*. Typhi** and ***S*. Paratyphi**) and **non-typhoidal (**e.g., ***S*. Choleraesuis**) Salmonellae.

Pathogenesis and Epidemiology

-- There are three types of ***Salmonella*** infections, **enterocolitis, enteric fevers** and **septicemia**.

-- **Enterocolitis** is characterized by an **invasion of the epithelial and subepithelial tissue** of the small and large intestine.

The organisms penetrate both through and between the mucosal cells into the lamina propria with resulting inflammation and diarrhea.

Neutrophil response limits the infection to the gut and the adjacent mesenteric lymph nodes; bacteremia is infrequent.

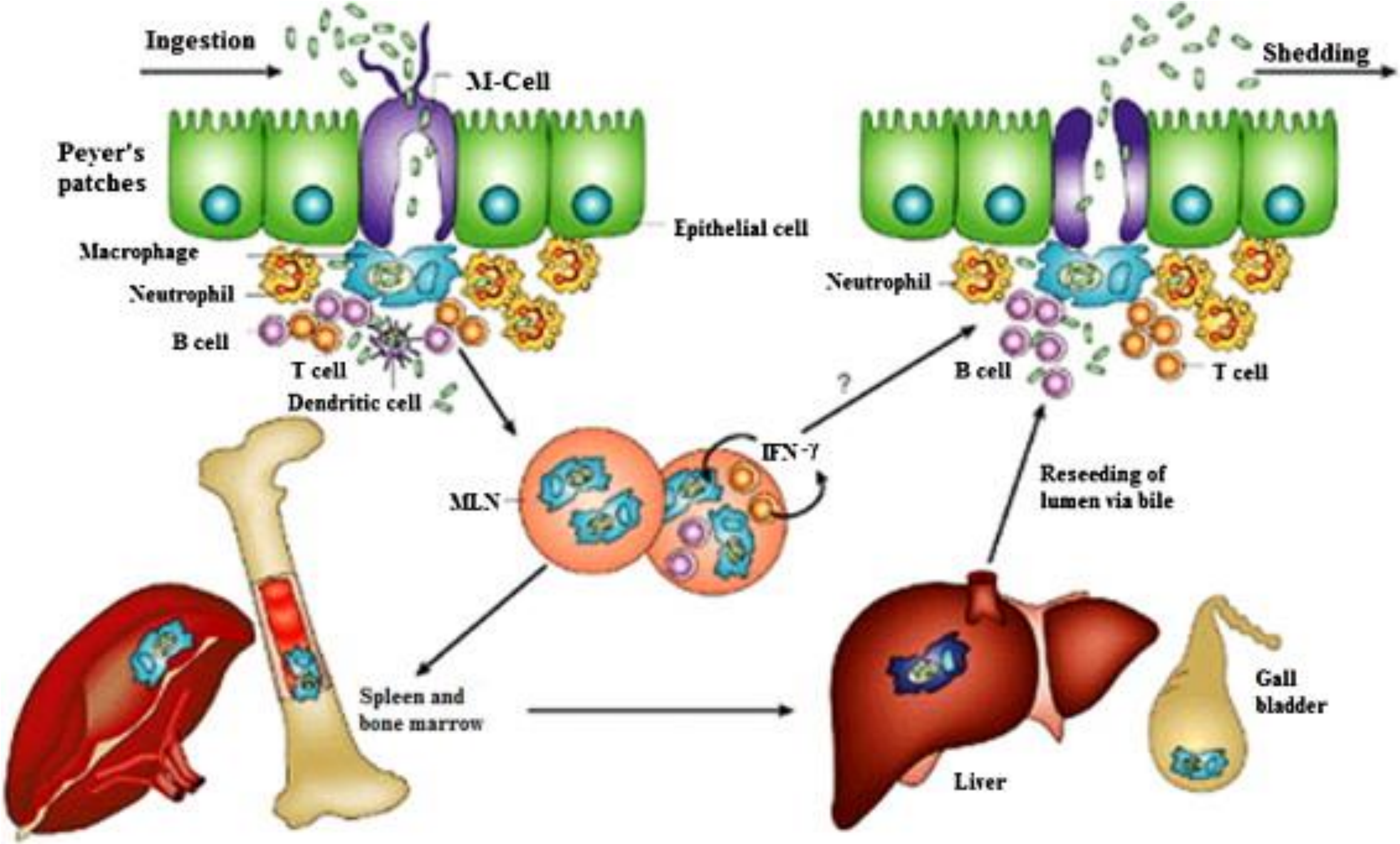
In contrast, ***Shigella*** enterocolitis need very small dose (**100 as compared to 100,000** organisms). Gastric acid is an important host defence.

-- In **enteric fevers**, infection **begins in the small intestine**, but few gastrointestinal symptoms occur.

-- **The organisms enter and multiply in the mononuclear phagocytes of Peyer's patches.**

-- They **spread to the phagocytes of liver, gallbladder and spleen leading to bacteremia** which is associated with the onset of fever and other symptoms, probably caused by endotoxins.

-- **Intestinal perforation** in the distal ileum is a very serious complication and is **frequently fatal**. It may occur without alarming symptoms until septicemia or diffuse peritonitis sets in.


Ingestion
M-Cell
Peyer's patches
Epithelial cell
Macrophage
Neutrophil
B cell
T cell
Dendritic cell
Shedding
Neutrophil
B cell
T cell
?
IFN-γ
MLN
Reseeding of lumen via bile
Spleen and bone marrow
Gall bladder
Liver

-- **Survival and growth of the organism within phagosomes in phagocytic cells is a striking feature of the disease.**

-- Another remarkable feature is the **preference for invasion of the gallbladder** which can result in establishment of the carrier state and excretion of bacteria in the feces for long periods.

-- **Septicemia** accounts for **only 5-10%** of Salmonella infections and occurs either in patients with an **underlying chronic disease** or a child with **enterocolitis**.

-- Septic course is slower than that with some other gram negative bacteria.

-- **Bacteremia leads to infection of many organs with osteomyelitis, pneumonia and meningitis as the most common sequelae.**

-- They infect **previously damaged tissues** and are also an important cause of **vascular graft infections**.

Epidemiology

-- It is mainly related to **ingestion of contaminated food and water.**

-- With exception of ***S*. Typhi** and ***S*. Paratyphi** which are **strict human pathogens**, others have a significant animal reservoir as well.

-- Human source are **carriers or patients who excrete the organisms** for a short period after infection.

-- Most common animal source is **poultry** and **eggs**, but inadequately cooked meat products are also important.

Clinical findings

Enterocolitis

-- After an incubation period of **12-48 hours**, **enterocolitis** begins with **nausea** and **vomiting** progressing to **abdominal pain** and **diarrhea**, which can vary from mild to severe, with or without blood.

-- Disease lasts for a few days, is **self limited**, cause **non-bloody diarrhea** and does not require medical care except in very young and very old.

-- **HIV infected persons** with **lower CD4 count** have greater severity of disease. ***S.* Typhimurium** is the most common cause.

Enteric fever

-- In **enteric fever** which is caused by ***S*. Typhi** and ***S*. Paratyphi A, B,** and **C**, the **onset is slow with fever and constipation** rather than vomiting and diarrhea as main symptoms.

-- **After first week, due to sustained bacteremia, high fever, delirium, tender abdomen and enlarged spleen occur.**

-- **Leukopenia and anemia are rarely seen. LFT are often abnormal indicating hepatic involvement.**

Enteric fever

-- Rose spots are specific but occur rarely.

-- **Disease begins to subside by third week but severe complications such as intestinal hemorrhage or perforation can occur.**

-- About **3% patients become chronic carriers** with higher rates in women.

-- **Septicemia** is often caused by ***S*. Choleraesuis**. There is fever but no enterocolitis.

-- There is further **progression to focal symptoms** associated with the affected organ, frequently bone, lung, or meninges.

Laboratory diagnosis

In **enterocolitis**, **stool culture** is preferable.

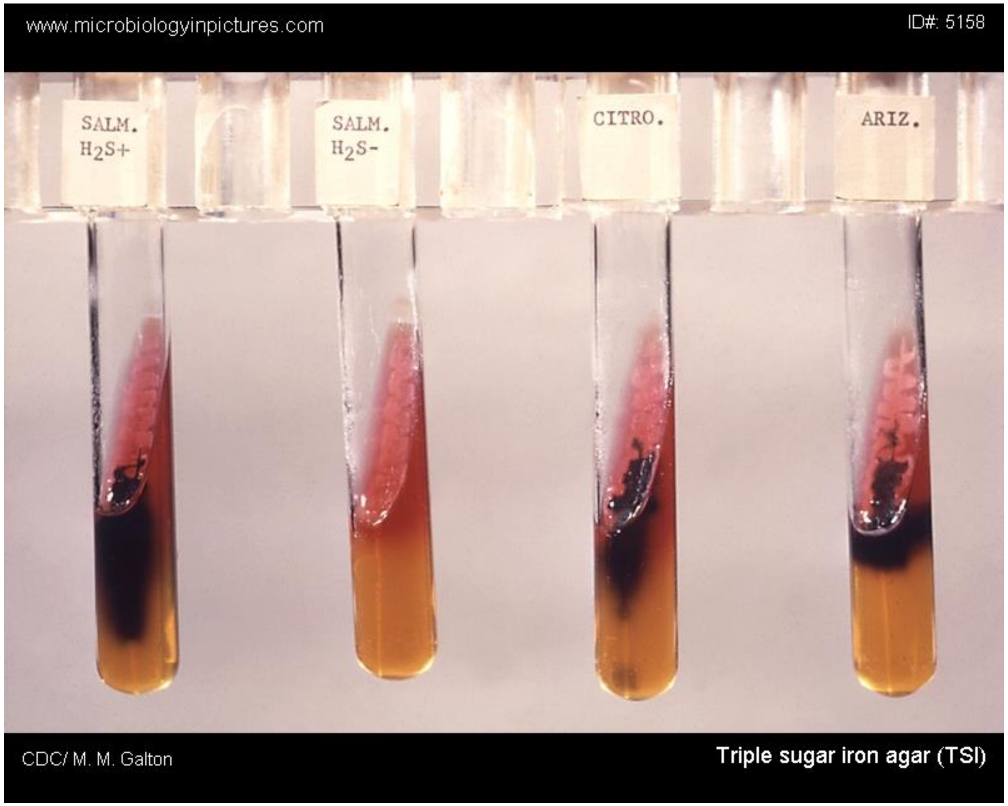
However, in **enteric fevers**, **blood culture** is the best option during first two week of illness.

Bone marrow cultures are often positive. Stool cultures may also be positive especially in chronic carriers.

Salmonellae form **non lactose fermenting colonies**.

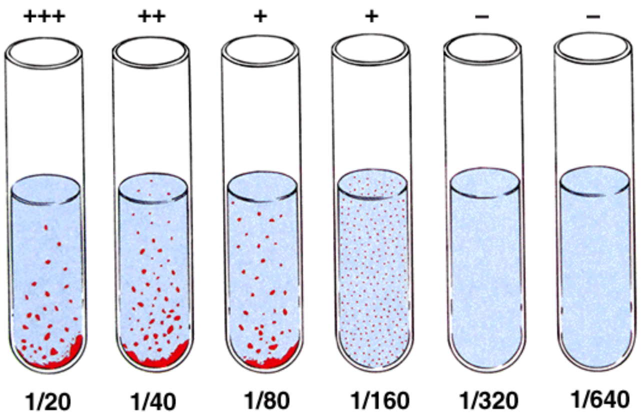
-- On **TSI**, an **alkaline slant and an acid butt, frequently with both gas and H_2S** are produced.

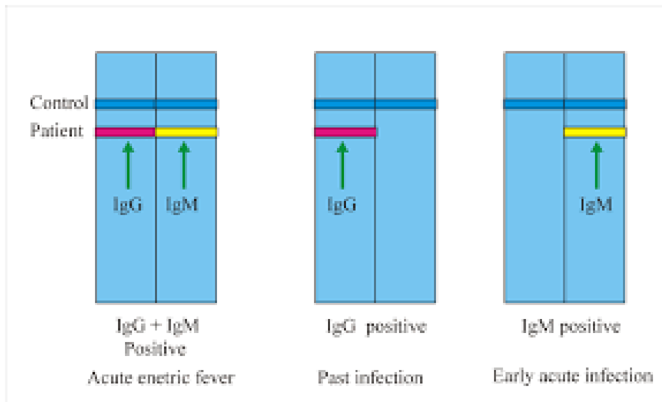
-- ***S*. Typhi** is a major exception. **It does not form gas and produce only a small amount of H_2S.**



-- **Differentiation from *Proteus* is made by urease activity** (negative in Salmonellae).

-- Serotyping is done on basis of O antigen. If organism is difficult to recover, serological tests such as **Widal test** or **Typhidot** are used.





Treatment

-- **Enterocolitis is self limiting** and only fluid and electrolyte replacement may be required.

-- Antibiotic treatment may prolong excretion, increase frequency of carrier state and select resistant mutants.

-- But **antimicrobial treatment is indicated for neonates or persons with chronic diseases** who are at risk for septicaemia and disseminated abscesses.

-- **For treatment of enteric fevers, ceftriaxone or ciprofloxacin are drugs of choice.**

-- To treat **chronic** carriers **ampicillin** may also be employed.

-- **Cholecystectomy** may be necessary to abolish the chronic carrier state.

-- Focal abscesses should be **drained surgically** whenever feasible.

Prevention

-- ***Salmonella*** infections are prevented mainly by public health and personal hygiene measures.

-- **Two vaccines** for **S. Typhi** are available, one contains **Vi polysaccharide** and other is a **live attenuated strain**.

-- A **conjugate vaccine** which has **more than 90% efficacy** and can be given to children will be shortly available.


Thank You!