Microbiology

Doctor 2017 | Medicine | JU

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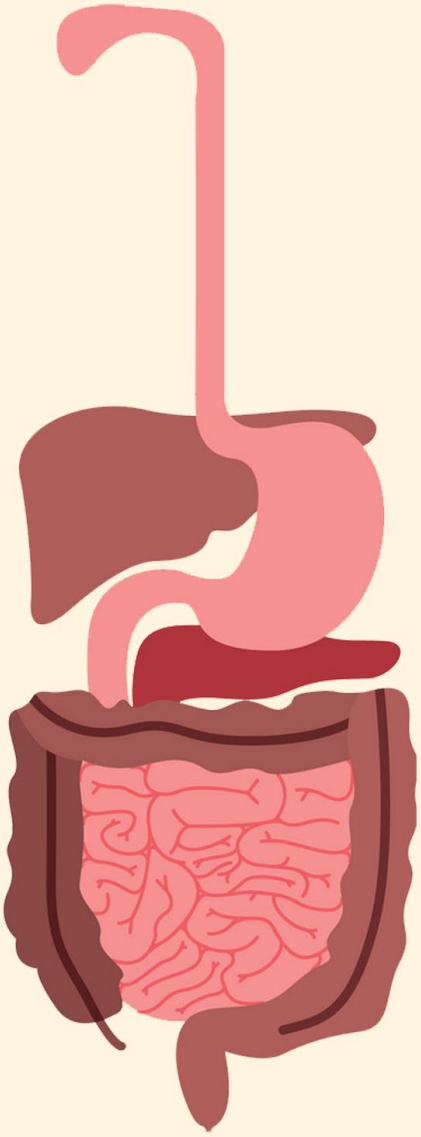


2nd system - GI

Outline:

- *We will be discussing gram-negative, facultatively anaerobic, fermentative, enteric bacteria:*
  1- ***Vibrios*** *(V. cholera), the causative agent of classic Cholera.*
  2- ***Campylobacters*** *(C. jejuni and C. coli), are invasive and cause bloody diarrhea.*
  3- ***Helicobacter pylori,*** *the causative agent of peptic ulcers.*
  4- ***Plesiomonas****. DNase positive in contrast with Aeromonas.*
  5- ***Aeromonas****. DNase negative.*
- *They are all oxidase positive possessing one polar flagellum (except H. pylori has multiple).*
- *The Vibrios (halotolerant), Plesiomonas and Aeromonas (not halotolerant) are aquatic bacteria.*

The Vibrios

- Vibrios are among the **most common** bacteria in **surface waters** worldwide; they are **aquatic** motile bacteria.
- Vibrio causes several important infectious syndromes. **Classic** among them is **Cholera**, a devastating **watery** diarrheal disease caused by **Vibrio cholera** that has been responsible for seven global pandemics and much suffering over the past two centuries and remains a significant public health concern in the developing world today.

Vibrios Species:

1- **V. cholerae** serogroups **O1** and **O139** cause **cholera epidemics**, they mainly contaminate **water surfaces** and are limited only to the GI tract *"what we will focus on"*.

2- **V. parahaemolyticus** is a marine bacterium that causes gastrointestinal diseases. It is the most common cause of **Sea-foodborne** *(raw fish or shellfish)* **gastroenteritis** in Asia.

3- **V. vulnificus**, found in **oysters**, causes severe **sepsis** in patients with **cirrhosis** and primary wound infection. *Vulnificus is Latin for "wound maker."*

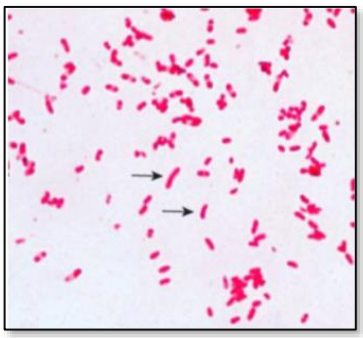
4- **V. alginolyticus** occasionally cause eye, ear, and wound **infections**.

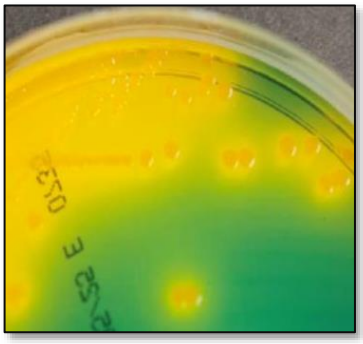
To sum it up:	*V. cholerae* → *Cholera*	*V. parahaemolyticus* → *Enteritis*
	V. vulnificus → *Sepsis*	*V. alginolyticus* → *Infections*

1- Vibrio Cholera

- The epidemiology and **spread of cholera** closely parallel the recognition of V. cholerae transmission in **water** and the development of sanitary water systems.

Characteristics:





- V. cholerae is a **comma-shaped**, curved rod 2–4 μm long. It is **motile** by means of a **polar flagellum**. On prolonged cultivation, Vibrios may become straight rods that resemble the gram-negative enteric bacteria.
- Characteristically, Vibrios grow at a **very high pH** (8.5 – 9.5) and are rapidly **killed** by acid; they cannot tolerate acid.
- V. cholerae produces convex, smooth, round colonies that are **opaque** and **granular** in transmitted light.
- V. cholera grows on thiosulfate-citrate-bile sucrose (**TCBS**) agar, a media **selective** for Vibrios, on which it produces **yellow** glistering colonies *(sucrose fermented)* that are readily visible against the dark-green background of the agar.
- A **positive oxidase** test result is a key step in the preliminary identification of Vibrios
- Vibrio species are **susceptible** to the compound **O/129** *(2,4-diamino-6,7diisopropylpteridine phosphate)*, which differentiates them from **other** species, which are **resistant** to O/129.
- All Vibrios are **halophilic**, requiring the presence of NaCl to grow, except V. cholera are **halotolerant** *(favors alkaline, acid sensitive);* NaCl often stimulates their growth.

Antigenic Structure and Biologic Classification:

1- H antigen:

- Many Vibrios share a single **heat-labile** flagellar **H antigen**. Antibodies to the H antigen are probably **not involved** in the protection of susceptible hosts.

2- O antigen:

- V. cholerae has **O** lipopolysaccharides that confer **serologic** specificity. There are at least **206** O-antigen groups forming different V. cholera strains. Antibodies to the O antigens tend to **protect** laboratory animals against infections with V. cholerae.
- V. cholerae strains of **O1** and **O139** can produce **cholera toxin** causing **classic cholera**, while **non-O1/O139** strains **can't** produce the toxin causing **cholera-like** diseases.
- V. cholerae **O1** has 2 **biotypes**, **classical** and **El Tor**. Each biotype is further subdivided into two **serotypes**, **Inaba Ogawa** and **Hikojima**.

Vibrio Cholerae Enterotoxin:

- **Cholera toxin**, a potent protein **enterotoxin** elaborated by the organism in the **small intestine** with a molecular weight (MW) of about 84,000, consisting of **'1' A and '5' B subunits**.
- The genes for V. cholerae enterotoxin are coded on the bacterial **chromosome**, not from a plasmid.
- Ganglioside **GM1** serves as the **mucosal receptor** for **subunit B,** which promotes **entry** of **subunit A** into the cell. Activation of subunit A1 yields increased levels of intracellular **cAMP**, thus resulting in prolonged **hypersecretion** of water and electrolytes.

Pathogenesis:

- Under natural conditions, V. cholerae is pathogenic **only** for **humans**. When a person with normal gastric acidity ingests V. cholera with **water**, **10^{10}** organisms or more are needed to become infected since it is **susceptible** to acid.
- If ingested with **food**, as few as **10^{2}-10^{4}** organisms only are necessary because of the **buffering** capacity of food protecting V. cholera from gastric acidity. Taking **antacids** and **PPIs** predisposes the body more to V. cholera *(low inoculum is enough to cause disease).*
- The toxin-coregulated pilus (**TCP**), so called because its synthesis is regulated in parallel with that of **cholera toxin**. It is **essential** for V. cholerae to **survive** and **multiply** in *(colonize)* the small intestine.
- The organisms do **not** reach the bloodstream but remain **within** the intestinal tract, i.e. they are not invasive.
- Virulent V. cholerae organisms attach to the **microvilli** of the brush border of epithelial cells. There they multiply and liberate **cholera toxin** and perhaps **mucinases** and **endotoxins**.

Clinical Findings:

- The burden of disease is often greatest during "cholera seasons" associated with **high** temperatures, heavy **rainfall**, and **flooding**, but cholera can occur year-round.
- About **50%** of infections with **classic** V. cholerae are **asymptomatic**, as are about **75%** of infections with the **El Tor biotype** of O1 strain.
- The incubation period is 12 hours - 3 days for those who develop symptoms, depending largely on the **size** of the inoculum that has been ingested.
- There is a sudden onset of **nausea** and **vomiting** and profuse **diarrhea** with abdominal cramps. Stools, which resemble "**rice water**," it contains mucus, epithelial cells, and Vibrios.

Note 1: *V. cholera (O1/ O139) cause a toxin-mediated* ***disease*** *through producing* ***cholera toxin****.* ***Colonization*** *occurs through producing the* ***toxin-coregulated pilus*** *(TCP).*

Note 2: ***O1 doesn't*** *form a* ***capsule****, while* ***O139 forms*** *one which contributes in pathogenesis.*

Diagnostic Laboratory Tests:

1- **Specimens**: mucus flecks from **stools** are used as specimens for culture.

2- **Culture**: growth is rapid in **peptone** agar, on **blood** agar with a pH near 9.0, or on **TCBS** agar. Typical colonies can be picked in 18 hours.

3- **Smears**: **Dark-field** or **Phase contrast** microscopy may show the rapidly **motile** Vibrios.

4- **Specific Tests**: V. cholerae organisms are further identified by slide agglutination tests using **O1 and O139-antisera** *(antibodies in the blood)* and by biochemical reaction patterns.

Treatment

- The most important part of therapy consists of aggressive **water and electrolyte replacement** to correct the severe dehydration, which if left untreated, it will cause death.
- Many **antimicrobial** agents are effective against V cholerae, but these play a **secondary** role in patient management. **Oral tetracycline** and **doxycycline** are drugs of choice that tend to reduce stool output in cholera and shorten the period of excretion of Vibrios.
- In children and pregnant women, alternatives to the tetracyclines include **erythromycin** and **furazolidone**.
- In some endemic areas, tetracycline resistance of V. cholerae has emerged; the genes are carried by transmissible plasmids.

Prevention

- Provision of safe water and of facilities for sanitary disposal of feces, improved nutrition, and attention to food preparation and storage in the household can significantly reduce the incidence of cholera.
- Currently, two oral killed **cholera vaccines** have been prequalified by the WHO and are available internationally:
  1- **WC-rBS:** contains several biotypes and serotypes of **killed** V. cholerae **O1** supplemented **with** recombinant cholera toxin B subunit.
  2- **BivWC:** contains several biotypes and serotypes of **killed** V. cholerae **O1 and O139 without** supplemental cholera toxin B subunit.

2- Campylobacter

- The classification of bacteria within the **family** Campylobacteriaceae changes frequently. Organisms with different genera have been added to this family, such as Helicobacter and Arcobacter.
- Campylobacters are **motile**, non-spore-forming, **curved**, gram-**negative** rods with a **polar flagellum** similar to Vibrios.
- Campylobacters natural reservoir is the **gastrointestinal tract** of many **animals** used for food *(poultry, cattle, sheep, and swine)* and many **household pets** *(birds, dogs, and cats).*
- Campylobacters are considered the commonest cause of bacterial self-limited enteritis. They cause both **diarrheal** and **systemic diseases**.

Campylobacter Species:

1- **C. jejuni:** the human pathogens fall into two major groups, those that primarily cause **diarrheal disease** and those that cause **extraintestinal** infection. **C. jejuni** is the prototype organism of the first group and is a very **common** cause of **diarrhea**.

2- **C. fetus:** it has two subspecies, fetus and venerealis. **C. fetus** subspecies fetus is an **opportunistic** pathogen that causes **systemic** infections in **immunocompromised** patients, however, it may occasionally cause **diarrhea**.

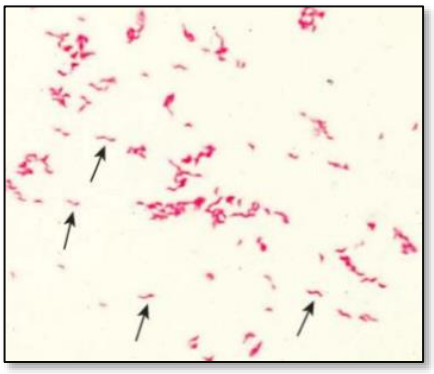
Other organisms that cause **diarrheal diseases** within the **family** Campylobacteriaceae include Campylobacter coli, Campylobacter upsaliensis, Campylobacter lari, Campylobacter hyointestinalis, Campylobacter fetus, **Arcobacter butzleri, Arcobacter cryaerophilus, Helicobacter cinaedi, and Helicobacter fennelliae.**

C. Jejuni and C. coli:

- Campylobacter jejuni and coli cause mainly **enteritis** and, occasionally, **systemic infection**. They are **invasive** causing bloody diarrhea.
- Both cause **infections** that are clinically **indistinguishable** and laboratories generally do not differentiate between the two species. Between 5% and 10% of infections reported to be caused by C. jejuni are probably caused by C. coli.
- Both are **thermophilic** (42°C) unlike other bacteria and are recovered from **stool only**, while **other** campylobacters can be recovered from the **blood**.
- C. jejuni colonizes the small intestine (jejunum) while C. coli colonizes the colon.

C. Jejuni:

- They are **gram-negative** rods with **comma**, S, or "**gull wing**" shapes. They are **motile**, with a single **polar flagellum**, and do not form spores.
- Selective media are needed, and incubation must be in an atmosphere with **reduced O2** and **added CO2** *(5% O2 and 10% CO2).*
- C. jejuni grows **well** at 36– 37°C. However, to **isolate** C. jejuni and **prevent the growth** of most of the **other** bacteria present in **feces**, incubation is done at **42°C** thus simplifying the identification of C. jejuni.



Pathogenesis of C. jejuni:

- The infection is acquired **orally** from food, drink, or contact with infected animals or animal products, especially **poultry**.
- C. jejuni is **susceptible** to **gastric acid**, and ingestion of about 10^4 organisms is usually necessary to produce infection.
- Both the **motility** of the strain and its capacity to **adhere** to host tissues appear to favor disease. However, Classic **enterotoxins** and **cytotoxins** *(cytolethal distending toxin 'CDT')* appear **not** to play substantial roles in pathogenesis; it is an invasive disease rather than a toxin-mediated disease.
- The organisms multiply in the **small intestine**, **invade** the epithelium, and produce inflammation that results in the **appearance** of red and white **blood cells** in the **stools**.
- Occasionally, the **bloodstream is invaded** *(unlike the Vibrios)* and a clinical picture similar to **enteric fever** develops. Localized **tissue invasion** coupled with the **toxic** activity appears to be responsible for **enteritis**.

Clinical Findings:

- A prodrome of **fever**, headache, myalgia, and/or malaise often occurs 12–48 h **before** the onset of **diarrheal symptoms**. Characteristically cause profuse **bloody** diarrhea.
- Usually, the illness is **self-limited** to a period of 5–8 days, but occasionally it continues longer.
- Most cases resolve **without** antimicrobial therapy; however, in about 5–10% of patients, symptoms may **recur**.

Complications:

1- In **immunocompromised** patients, rarely in others, **local suppurative infections** occur including cholecystitis, pancreatitis, and cystitis. **Distant complications** include meningitis, endocarditis, arthritis, peritonitis, cellulitis, and septic abortion.

2- Complications of **acute infections** include hepatitis, interstitial nephritis, and **hemolytic uremic syndrome.**

3- Certain serotypes of C. jejuni have been associated with post-diarrheal **Guillain-Barré syndrome**, a form of **ascending** paralytic disease. **Reactive arthritis** and **Reiter's syndrome** may also follow acute campylobacter diarrhea.

Diagnostic Laboratory Tests:

1- **Specimens**: **Diarrheal stool** is the usual specimen. C. jejuni, C. fetus, and other campylobacters may occasionally be recovered from **blood** cultures usually from **immunocompromised** or elderly patients.

2- **Smears**: **Gram-stained** smears of stool may show the typical "**gull wing**" shaped rods. **Dark-field** or **phase contrast** microscopy may show the typical **darting motility** of the organisms.

3- **Culture**: Culture on the selective media (Skirrow's, Butzler's, Blaser's, Campy-BAP and Preston media) is the definitive test to diagnose C. jejuni enteritis. If other species of Campylobacter are suspected other than C. jejuni, a medium **without** a **cephalosporin** should be used and incubated at **36–37° C**.

Treatment:

- Typically, it is a self-limited disease. First line therapy: fluids and electrolyte replacement.
- Even among patients presenting for medical attention with Campylobacter enteritis, **not al**l clearly, benefit from **antimicrobials therapies**. Indications for therapy include **high fever**, **bloody** diarrhea, **severe** diarrhea, **persistence** for >1 week, and **worsening** of symptoms. A 5- to 7-day course of **erythromycin** is the regimen of choice.

  An alternative regimen for adults is **ciprofloxacin** or another **fluoroquinolone** for 5–7 days.
- For **systemic infections**, treatment with **gentamicin**, **imipenem** or **chloramphenicol** should be started empirically, but susceptibility testing should then be performed.

3- Helicobacter Pylori

- H. pylori are **spiral-shaped** gram-**negative** rods. They have **multiple** flagella at **one** pole *(benefit in reaching deeper less acidic mucus*) in contrast to other mentioned bacteria where they possess **only one flagellum.** They have **cork-screw** motility.
- The organism has several **acid-resistance mechanisms**, most notably a highly expressed **urease** that catalyzes urea hydrolysis to produce buffering ammonia. It is also **microaerophilic** *(requires low levels of oxygen)*, **oxidase** and **catalase positive**, slow-growing, and requires complex growth media in vitro.
- H. pylori are associated with antral gastritis, duodenal ulcers, gastric ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue *(MALT)* lymphomas.
- It affects the absorption of vitamin B12 causing **Pernicious anemia** and predisposes some patients to **iron deficiency** through **occult blood** and **reduced iron absorption**.

> ***Note:*** *Pernicious anemia is a **decrease in red blood cells** that occurs when the intestines cannot properly absorb **vitamin B12.***

Epidemiology

- Helicobacter pylori **colonize the stomach** in ~50% of the world's human population, essentially for life unless eradicated by antibiotic treatment. Transmission is **Feco-Orally.**
- Humans are the **only** important **reservoir** of H. pylori. Children may acquire the organism from their parents *(most often the primary caregiver)* or from other children.
- Most H. pylori-colonized persons do **not** develop clinical importance. Whether the disease shows in someone or not depends on a **combination of factors**:

  Bacterial strain differences **(cag-positive), type IV** secretion system, the vacuolating cytotoxin A *(***VacA***)*, host **susceptibility** to disease, and **environmental factors** *(IL-1 gene polymorphisms, and smoking).*

Pathogenesis

- H. pylori are found deep in the **mucous layer** near the epithelial surface. They overly **gastric-type** but **not** intestinal- type epithelial cells.
- H. pylori are quite **motile**, even in mucus, and can find their way to the epithelial surface.
- H. pylori produce:

  1- A **protease** that modifies the gastric mucus and further **reduces** the ability of acid to diffuse from the mucus.

  2- A **potent urease**, which yields production of ammonia further **buffering** of gastric acid.

- The mechanisms by which H pylori causes' mucosal inflammation and damage are not well defined but probably involve **both** bacterial and host factors.
- The bacteria invade the epithelial cell surface to a **limited degree**. Toxins, LPS and the ammonia produced by the urease activity may also directly **damage** the mucosal cells.
- Polymorphonuclear and mononuclear cell **infiltrates** are seen within the **epithelium** and **lamina propria**, this causes **acute gastritis** which on the long term becomes **chronic gastritis**.
- **Vacuoles** within cells are often distinct, **destruction** of the **epithelium** is common, and **glandular atrophy** may occur. Thus, predisposing to **gastric cancer.**

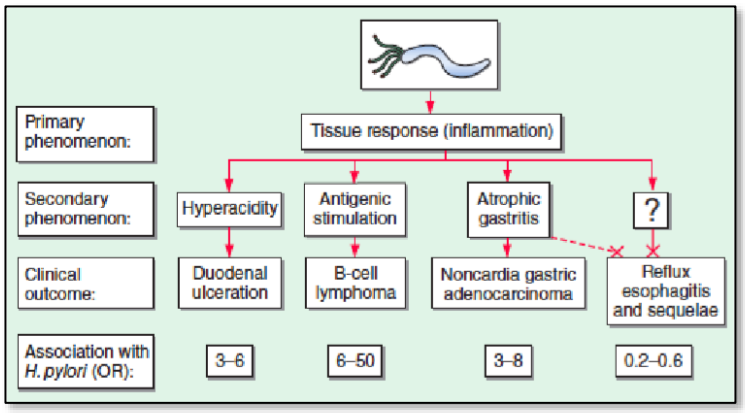
Clinical Findings of H. pylori:

- Acute infection can yield an **upper gastrointestinal illness** with nausea and pain, vomiting and **fever** may also be present. The acute symptoms may last for 1-2 weeks.
- After colonization, the H pylori infection persists for years, decades or even a **lifetime**; it is not transient. H. pylori infection is associated with about **90%** of patients with **duodenal ulcers** and **50–80%** of those with **gastric ulcers**. Recent studies confirm that H. pylori are a risk factor for **gastric adenocarcinoma** and **lymphoma**.

- Relationships were found between colonization with H. pylori and diseases of the upper gastrointestinal tract. For example:

  a- H. pylori colonization **increases** the risk of developing ulcers and gastric cancer.

  b- It is found to be **protective** against reflux esophagitis (**GERD**) as well as **esophageal carcinoma**.



Diagnostic Laboratory Tests: *(most used tests are non-invasive)*

1- **Smears:** A gastroscopy procedure with **biopsy** is required. **Routine stains** reveal **gastritis**, while **Giemsa** or **special silver** stains can show the curved or spiral-shaped **organisms**.

2- **Culture:** It is performed when patients are **not responding** to treatment, and there is a need to assess **susceptibility patterns**.

3- **Special non-invasive test:** Rapid tests to detect **urease activity** in vitro are widely used for presumptive identification of H. pylori in specimens.

   In vivo tests for urease activity are done such as **urea breath tests**. 13-C or 14-C labeled urea is ingested by the patient. If H. pylori are present, the urease activity generates labeled CO^2 detected in the patient's **exhaled breath**.

4- Detection of **H. pylori antigen** in **stool** specimens confirms **eradication** of H. pylori after treatment.

Treatment: *(antimicrobials, 2 at least, and acid target drugs)*

- **Triple antibiotics therapy** with metronidazole + bismuth subsalicylate/bismuth subcitrate + amoxicillin/tetracycline for 2 weeks **eradicates** H. pylori infection in 70–95% of patients.
- Erythromycin shows resistance in some cases.
- An **acid-suppressing agent** given for 4-6 weeks enhances **ulcer healing**, e.g. Proton pump inhibitors (**PPIs**) directly inhibit H. pylori and appear to be potent urease inhibitors.
- The preferred initial therapy is 7–10 days of either PPI plus amoxicillin and clarithromycin, or a quadruple regimen of a PPI, metronidazole, tetracycline, and bismuth for 10 days, resulting in **complete eradication**.

4- Plesiomonas

- Un-commonly, Plesiomonas have been associated with **diarrheal disease** in humans.
- **Plesiomonas shigelloides** is an **oxidase positive**, gram-negative rod with **polar flagella**.
- Plesiomonas is most common in **tropical** and **subtropical** areas. It is a **water** and **soil** organism and has been isolated from **freshwater** fish and many animals; it is **not** halotolerant, unlike the Vibrios.
- Most isolates from **humans** have been from **stool cultures** of patients with **diarrhea**.
- Plesiomonas **grows** on the differential media used to isolate Salmonella and Shigella from **stool** specimens but not on TCBS.
- Some Plesiomonas strains **share antigens** with **Shigella sonnei**, and **cross-reactions** with **Shigella antisera** occur. Plesiomonas can be **distinguished** from **shigella** in **diarrheal stools** by the **oxidase test**: **Plesiomonas** are oxidase **positive** while **shigella** are **not**.
- **Plesiomonas** species are **positive** for **DNase**; this and other biochemical tests distinguish it from Aeromonas species.
- They are **resistant** to O/129 compound which differentiates it from V. cholera *(recall page 2)*.

5- Aeromonas

- Aeromonas species are distinguished from:
  1- **Enteric gram-negative rods,** by finding a **positive oxidase** reaction in growth obtained from a blood agar plate.
  2- **Vibrios,** by showing **resistance** to the compound **O/129**, like Plesiomonas, and **lack** of **growth** on media containing 6% **NaCl**.
  3- **Plesiomonas,** by being **DNase negative**.
- **Typically**, aeromonads produce **hemolysins**. Some strains produce an **enterotoxin**, **cytotoxins** and the ability to **invade cells** in tissue culture.
- **Gastroenteritis** is caused mostly by **Aeromonas caviae complex**, it ranges from acute **watery** diarrhea to less commonly a **dysenteric** type *(bloody diarrhea).*
- Aeromonas species are also associated with **extraintestinal infections** such as **bacteremia** and **wound infections**. The latter is often the result of **trauma** that occurs in a **water** environment and is caused primarily by **Aeromonas hydrophila**.

Treatment

- **Aeromonas** and **Plesiomonas** species are generally **susceptible** to fluoroquinolones (ciprofloxacin), third and fourth generation cephalosporins, carbapenems, and aminoglycosides, but **resistance** has been noted to all those agents. Because **Aeromonas** can produce various **β-lactamases**, including **carbapenemases**.

Good Luck ♥

"This is just to recap what was taken, it is not everything."

Bacteria	Characteristics	Pathogenicity	Diagnosis	Notes
Vibrio Cholera O1 and O139 *Marine bacteria*	- Comma-shaped. - Motile with a polar flagellum. - Grows in a very high pH. - Halotolerant. - Sucrose fermenter.	- Cholera toxin *(A and B subunit)*, GM1 serves as a mucosal receptor. - TCP to survive and multiply. - Does not reach the bloodstream. - 'Rice stool'.	- Yellow colonies on TCBS. - Grows on peptone/blood agar. - Anti-O1/O139 antigens. - Susceptible to O/129.	- Attach to the microvilli of brush border. - **Vaccines**: Wc-rBS and BivWC from O antigen, H antigen does not protect.
Campylobacter *Bloody diarrhea*	- Curved, darting motility, with a polar flagellum. - **C. jejuni:** gull wing, grows in isolation at 42°. - Usually self-limited.	- C. jejuni and coli cause enteritis mainly. C. fetus causes systemic infections mainly. - Invades bloodstream. - Prodrome fever followed by diarrhea.	- Diarrheal stool or blood from immunocompromised as specimens. - Skirrow's, Butzler's, Blaser's, Campy-BAP and Preston media for C. jejuni only.	- Main reservoirs are animals (poultry) and pets. - Multiply in S. intestine. - Associated with **Guillain-Barré syndrome** and HUS.
H. Pylori *Peptic ulcers*	- Spiral-shaped, and microaerophilic. - Cork-screw motility with **multiple** flagella at one pole.	- Affects iron and B12 absorption (pernicious anemia). - Risk factor for gastric cancer MALToma, chronic gastritis and peptic ulcers.	- Catalase positive. - Acid resistance (protease, urease). - Giemsa, silver stains and urease breath test. - Antigens in stool confirm eradication.	- Humans are the primary reservoir, colonizing gastric epithelial cells. - **Tx**: 3 ABs + PPIs. - Has a **type IV** secretion system.
Plesiomonas *Marine bacteria*	- Polar flagellum. - Found in fresh water.	- Diarrheal diseases.	- Resistant to O/129. - DNase positive.	- Cross-reactions with Shigella antisera.
Aeromonas *Marine bacteria*	- Polar flagella.	- Produce hemolysins. - Diarrheal diseases.	- Resistant to O/129. - DNase negative.	- Gastroenteritis is caused mostly by Aeromonas caviae complex, wound infections by Aeromonas hydrophilia.