GIS 6

MICROBIOLOGY

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In this sheet we will be discussing gram-negative, facultatively anaerobic, fermentative and enteric bacteria:

1- Vibrios (V. cholera), the causative agent of classic Cholera.
2- Campylobacters (C. jejuni and C. coli), the commonest cause of bacterial enteritis in the developed world.
3- Helicobacter pylori, the causative agent of peptic ulcers.
4- Plesiomonas.
5- Aeromonas.

[Note: the information from slides that are not mentioned by the doctor are highlighted in green and put between brackets].

❖Vibrio

- Vibrios are among the most common bacteria in surface water worldwide, they are called **aquatic bacteria**.
  Note:They are found in saline water, in contrast to Plesiomonas and aeromonas which live in fresh water.
- Vibrios cause a number of important infectious syndromes. Classic among them is cholera, a devastating diarrheal disease caused by Vibrio cholerae.
- They are motile, possess polar flagella (heat labile flagellar H antigen), [Antibodies to the H antigen are probably not involved in the protection of susceptible hosts.]
- They are gram-negatives, so they possess O antigen, and they are classified to serotypes according to the O antigen.

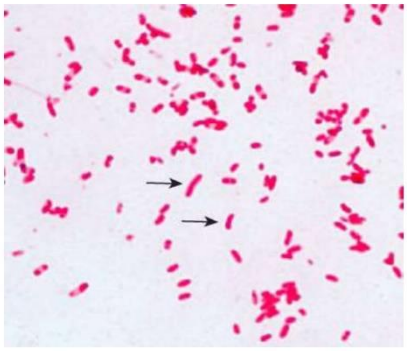
➢ Vibrios species:

1) V cholerae serogroups **O1** and **O139**, they are the only ones able to cause **epidemics and pandemics** of cholerae. (IMPORTANT)
   The O1 serotype is further classified into 2 biotypes: El Tor, Classical.
   Each biotype is further subdivided into 3 serotypes: Inaba, Ogawa, hikojima.
   Note: El Tor is the biotype that is circulating nowadays.
2) Other serogroups include **Non-O1** and **Non-O139**, these are associated with **cholerae like disease**, which is a form of mild gastroenteritis.
3) **vibrio parahaemolyticus**, causes gastroenteritis, and it's commonly associated with sea food (raw fish or shellfish) especially in South East Asia, Japan and China.
4) **Vibrio vulnificus**, found in oysters, causes sepsis in patients with cirrhosis and primary wound infections. Vulnificus is Latin for "wound maker."

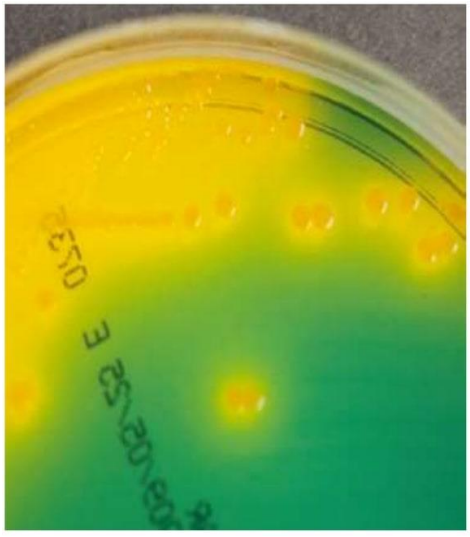
Note: O1 doesn't have capsule, while O139 have a polysaccharide capsule.

- Vibrios are considered halotolerant, which means they **can tolerate** a certain concentration of sodium chloride, and some of them like parahaemolyticus are halophilic meaning that they **require** sodium chloride for their growth.
- The reservoir is feces from infected patients or asymptomatic carrier patients.
- The transmission is by contaminated water and food (feco-oral route).
- Note that V. **cholerae** is from contaminated food and water whereas V. **parahaemolyticus** is mainly from food.

- V cholerae is a comma-shaped, curved rod 2–4 µm long, possesses one polar flagellum, usually stains faintly on gram stain. [On prolonged cultivation, vibrios may become straight rods that resemble the gram-negative enteric bacteria.]



- TCBS (Thiosulfate-citrate-bile salts-sucrose agar) is the **selective agar** used to isolate V.cholerae, on which it produces glowy yellow colonies with halos around them (sucrose fermented) that are readily visible against the dark green background of the agar. The colony can extend up to 1 cm (this is unusual with other bacteria).
- [V cholerae produces convex, smooth, round colonies that are opaque and granular in transmitted light.]
- Note: One of the characteristic features of vibrio is that they can ferment a variety of sugars, one of them is sucrose that gives these yellow colonies.



- Vibrios **tolerate high Ph** (8.5-9.5), and we exploit this feature when we suspect V.cholerae, by putting them on peptone alkaline water which will favor the growth of vibrios and inhibit the growth of other bacteria.
- They are **acid sensitive**, they get killed by the acidity of the stomach, that's why we need **large inoculum** to establish the infection.

Note: the inoculum size is also dependent on the way of infection, if by contaminated water → large inoculum (as many as 10^{10}), contaminated food → lower inoculum (as few as 10^2-10^4) because the food will buffer the acidity of the stomach.
Also, if the infected patient is on drugs like PPI or H2 blockers, or is suffering from achlorhydria (reduced acidity of the stomach), he will be more prone to establish an infection with vibrio.

- Vibrios are oxidase positive, and this feature differentiates them from Enterobacteriaceae.
- Vibrio species are susceptible to the compound O/129 (2,4-diamino-6,7-diisopropylpteridine phosphate), which differentiates them from Aeromonas and plesiomonas species, which are resistant to O/129.
(this differentiation is important since these species live in water as well as vibrios)

➢ Pathogenicity

- Vibrio cholerae produces an enterotoxin that causes cholera (toxin mediated disease), profuse watery diarrhea that can rapidly lead to dehydration and death.
- Cholera toxin is an AB toxin, it has one A (active) subunit, and five B (binding) subunits.
- B subunits bind to the receptors on gangliosides (GM1 receptor), once they bind, the A subunit is internalized into the cell, which acts on G stimulatory proteins and activates adenylyl cyclase, increasing cAMP, resulting in the hypersecretion of fluid and electrolytes.
Note: it's the same mechanism of heat labile enterotoxin in ETEC, also there is cross reactivity between these two toxins, so being infected with ETEC can stimulate the production of antibodies against cholera toxin.
- **The toxin-coregulated pilus** (TCP), so named because its synthesis is regulated in parallel with that of cholera toxin (located on the same pathogenicity island), is an essential virulence factor for V. cholerae to survive and multiply (colonize) in the small intestine.
- The organisms do not reach the bloodstream but remain within the intestinal tract.
- 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 **endotoxin**.

➢ 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.
- The diarrhea caused by cholera is very severe watery diarrhea with fluid loss of one liter per hour (the patient can lose more than 20 liters in a day), which will lead to dehydration, circulatory collapse, anuria, metabolic acidosis (due to loss of HCO3), hypokalemia (loss of K), cardiac arrythmias and might lead to sudden death (Mortality rate = 50%).
- There is a sudden onset of nausea, vomiting and profuse watery painless diarrhea with abdominal cramps. Stools, which resemble "**rice water**", contain mucus, epithelial cells, and large numbers of vibrios.
- [The incubation period is 12 hours–3 days for people who develop symptoms, depending largely on the size of the inoculum ingested.]
- [About 50% of infections with classic V.cholerae are asymptomatic, as are about 75% of infections with the El Tor biotype.]
- [The diagnosis of a full blown case of cholera presents no problem in the presence of an epidemic. However, sporadic or mild cases are not readily differentiated from other diarrheal diseases. The El Tor biotype tends to cause milder disease than the classic biotype.]

➢ Diagnostic laboratory tests

- Specimens for cultures consist of mucus flecks from stools.
- Smears

  Dark field or phase contrast microscopy may show the rapidly motile vibrios.
- Culture

  Growth is rapid in alkaline peptone agar (8-9 PH), or blood agar with a pH near 9. Then we subculture them on TCBS agar (selective), and typical colonies can be picked in 18 hours.
- Specific tests

  V. cholerae serotypes are further identified by slide agglutination tests using anti-O group 1 or group 139 antisera and by biochemical reaction patterns.

➢ Treatment

- ✓ The most important part of therapy consists of water and electrolyte replacement to correct the severe dehydration and salt depletion. (FIRST LINE TREATMENT)

- ✓ Many antimicrobial agents are effective against V cholerae, but they play a secondary role in patient management. Oral tetracycline (doxycycline)-100 mg/ day for 5-7 days tend to reduce stool output in cholera and shorten the period of excretion of vibrios.

  Note: tetracyclines are contraindicated in pregnant women and lactating women, and in children under the age of 9, thus we give them erythromycin with the water and electrolyte replacement.

  [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.
- ✓ The patient should be isolated, the excreta should be disinfected, with following up with his contacts.

- ✓ Currently, there are two oral heat-killed cholera vaccines:
  1) **WC-rBS (Sweden)** contains several biotypes and serotypes of V. cholerae O1 supplemented with recombinant cholera toxin B subunit. Recommended by the WHO.

  2) **BivWC (India)** contains several biotypes and serotypes of V. cholerae O1 and V. cholerae O139 without supplemental cholera toxin B subunit.

  Unfortunately, these vaccines have very limited protection, the protection percentage dropped in the second year of vaccination to zero.

  3) Recently in 2016 a new vaccine has been developed called **Vaxchora**, a live attenuated vaccine, contains O1 serotype (inaba).

  Note: all of these vaccines are given orally.

❖campylobacter

- Campylobacters are motile, non-spore-forming, curved, gram-negative rods.
- They are one of the differentials of invasive bacteria that cause bloody diarrhea (WBCs RBCs and mucus).
- The **commonest** cause of bacterial enteritis in the **developed** world.
- The reservoir is the gastrointestinal tract of many animals used for food (including poultry, cattle, sheep, and swine) as well as the dairy products [and many household pets (including birds, dogs, and cats).]
- Infection with campylobacter confer a certain degree of immunity. They noticed that the infection to disease ratio falls with age (the older the patient gets, the less prone he is to develop campylobacter infection).
- The human pathogens fall into two major groups: those that primarily cause diarrheal disease and those that cause extraintestinal infection.

➢ Campylobacters species

- Campylobacter jejuni and coli have emerged as common human pathogens, causing mainly enteritis and occasionally systemic infections.
  - ✓ C. jejuni is the prototype organism in the group and is a very common cause of diarrhea in humans.
  - ✓ C. jejuni and C. coli cause infections that are clinically indistinguishable, and laboratories generally do not differentiate between the two species.
  - ✓ Usually, C. jejuni infects the jejunum, whereas C. coli infects the colon.
  - ✓ [Between 5% and 10% of infections reported to be caused by C jejuni are probably caused by C coli.]

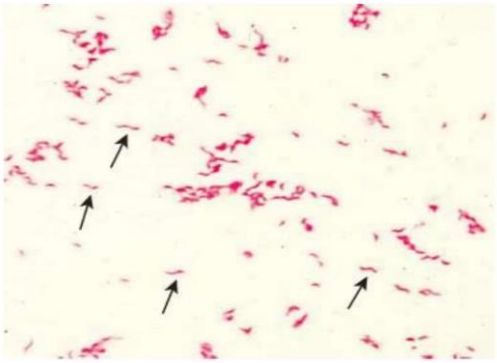
- Campylobacter fetus and venerealis: associated with extraintestinal manifestations. Common among immunocompromised patients (opportunistic) and it's an invasive infection, but how invasive it is and the limit of the invasion depends on the host factors and the bacterial factors. C. fetus causes systemic infection in immunocompromised patients and may occasionally cause diarrhea.

- Helicobacter cinaedi, and Helicobacter fennelliae.
  Note: these are intestinal bacteria unlike the helicobacter pylori that lives only in the stomach.
- Campylobacter lari, mostly isolated from seagulls.
- Campylobacter upsaliensis, mostly isolated from dogs.
- [Campylobacter hyointestinalis]
- [Campylobacter butzleri]

- [Arcobacter cryaerophilus]

- [The classification of bacteria within the family Campylobacteraceae has changed frequently. Some species previously classified as campylobacters have been reclassified in the genus Helicobacter. The genus Arcobacter has been created.]

- gram-negative rods with comma, S, or "**gull wing**" shapes.
- They are motile, with a single polar flagellum, and do not form spores. Their motility is called darting motility (quick movement).
- They grow well at 42 °C. Although C. jejuni grows well at 36–37°C, incubation at 42°C prevents growth of most of the other bacteria present in feces, thus simplifying the identification of C. jejuni. Several selective media are in widespread use.
- They are microaerophilic (require little oxygen), and they grow best at 5% O2 with added 10% CO2.



➢ Pathogenesis

- It's similar to salmonella and shigella, invasive intracellular infection, bloody diarrhea with RBC, WBC and mucus.
- They produce enterotoxins and cytotoxins (cytolethal distending toxin CDT), but these toxins play a secondary role in the pathogenesis (the main is invasion).
- 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, and a clinical picture similar to enteric fever develops.]
- [C. jejuni is susceptible to gastric acid, and ingestion of about 104organisms is usually necessary to produce infection.]

➢ Clinical findings

- The incubation period is less than a week followed by a prodrome of fever, headache, myalgia, and/or malaise often occurs 12–48 h before the onset of diarrheal symptoms, profuse diarrhea that may be grossly bloody.
- Usually the illness is self-limited to a period of 5-8 days, but occasionally, in severe cases, it can last up to two weeks.

- Campylobacter infection can trigger Reiter's syndrome (reactive arthritis with conjunctivitis, polyarthritis and urethritis).
- They are also associated with Guillain-Barre syndrome, acute debilitating disease, form of ascending paralytic disease, because of developing antibodies against neuronal epitopes after Campylobacter infection.

  Note: The previous two syndromes may develop after 1 month of the disease.
- Immunocompromised people or those with malignancies or AIDS might suffer from a lot of complications such as cholecystitis, pancreatitis, and cystitis; distant complications include meningitis, endocarditis, arthritis, peritonitis, cellulitis, and septic abortion.
- [Hepatitis, interstitial nephritis, and the hemolytic-uremic syndrome occasionally complicate acute infection.]

➢ Diagnostic laboratory tests

○ 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.]

○ 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.

○ Culture

42 °C microaerophilic media, or the use of selective media (Skirrow's, Butzler's, Blaser's, Campy-BAP and Prestonmedia) to diagnose C. jejuni infection.

[If another species of Campylobacter is suspected, medium without a cephalosporin should be used and incubated at 36–37°C.]

✓ Nowadays there are immunochromatographic assays, once they detect the antigen of campylobacter in the stool, they change the color and give a quick result.

➢ Treatment

- Fluid and electrolyte replacement is central to the treatment of diarrheal illnesses.
- when there is invasion, you should prescribe antibiotics, but the exception with campylobacter is that antibiotics play a secondary role (not all patients clearly benefit from specific antimicrobial therapy), though you should take the age of the patient and the severity of the disease into consideration. 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 or Azithromycin (regimen of choice).
- [An alternative regimen for adults is ciprofloxacin or another fluoroquinolone for 5–7 days.]
- [For systemic infections, treatment with gentamicin or imipenem or chloramphenicol should be started empirically, but susceptibility testing should then be performed.]

✓ Prevention involves proper food preparation and avoidance of unpasteurized milk.

❖Helicobacter pylori

- H pylori is **a spiral-shaped** gram-negative rod, they can live in the stomach with its acidity (remarkable)
- It has **multiple flagella** at one pole and is actively motile. They have cork-screw motility.
- The only species in its genus. They're the causative agent of peptic ulcer (gastric & duodenal)
- H. pylori is microaerophilic, oxidase positive and catalase positive, slow-growing, and requires complex growth media in vitro.
- Transmission is feco-oral, from the food contaminated with the feces of infected patients or asymptomatic carriers.
- H pylori is associated with antral gastritis, 85% of gastric ulcers, 95% of duodenal ulcers, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphomas.
- [It may be an initial precipitant of pernicious anemia, it may also predispose some patients to iron deficiency through occult blood loss and/or hypochlorhydria and reduced iron absorption.]
- H pylori colonization has been found to be protective against GERD disease and esophagus adenocarcinoma.

- Helicobacter pylori colonizes the stomach in ~50% of the developed world, and more than 80% of the developing world. H pylori colonization is essential for life (lifelong) unless eradicated by antibiotic treatment.
- [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 people do not develop clinical sequelae. That some people develop overt disease whereas others do not is related to a combination of factors: bacterial strain differences (cag-positive, type IV secretion system, the vacuolating cytotoxin VacA), host susceptibility to disease, and environmental factors (the interleukin 1 gene polymorphisms, and smoking).]

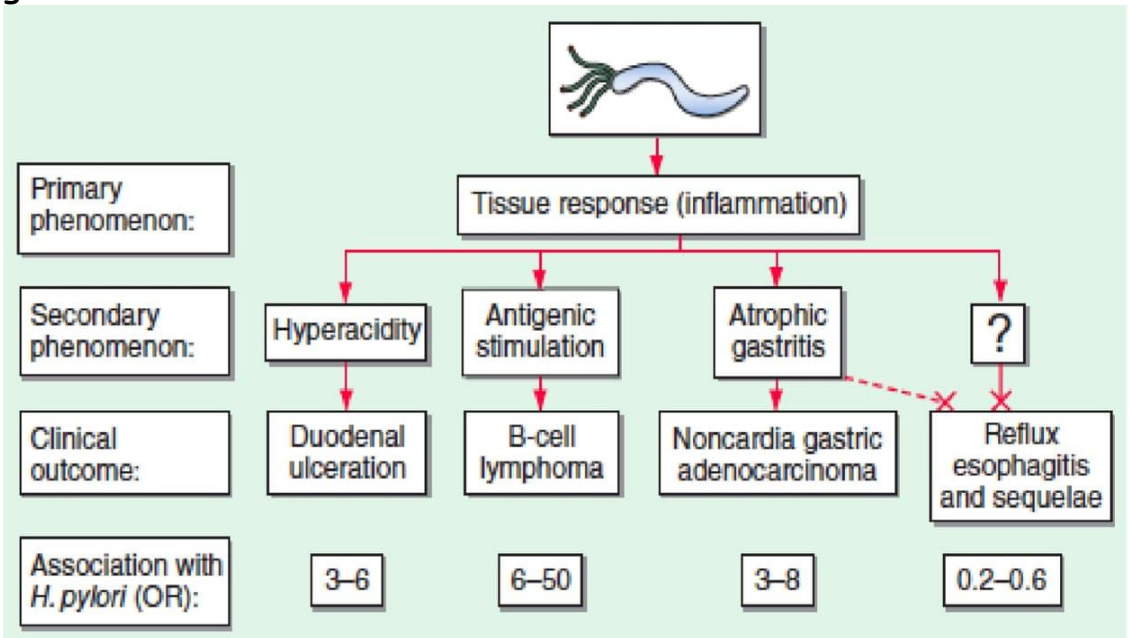
➢ Pathogenesis

- H pylori is found deep in the mucous layer near the epithelial surface where physiologic pH is present.
- H pylori is quite motile, even in mucus, and is able to find its way to the epithelial surface. H pylori overlies gastric-type but not intestinal-type epithelial cells.
- H pylori also produces a protease that modifies the gastric mucus and further reduces the ability of acid to diffuse through the mucus.
- The organism has several acid-resistance mechanisms, most notably a highly expressed **urease** that catalyzes urea hydrolysis to produce buffering ammonia.
- It produces two toxins: Cag-A (cytotoxin associated gene), Vac-A (vacuolating cytotoxin).
- It causes acute gastritis with huge infiltration of mononuclear and polymorphonuclear cells. On the long run, it can cause chronic gastritis, ulcers, adenocarcinoma, fibrosis and metaplasia of the cells lining the stomach.
  Refer to slide 26 for more details.

➢ Clinical findings

Acute infection can yield an upper gastrointestinal illness with nausea and gastric pain, burning sensation, vomiting and fever may also be present. The acute symptoms may last for less than 1 week or as long as 2 weeks.

✓ The schematic representation below shows the relationships between colonization with Helicobacter pylori and diseases of the upper gastrointestinal tract:



Notice that the odds ratio for duodenal ulcers, B-cell lymphoma and gastric adenocarcinoma is more than 3, which indicates the strong correlation between H pylori infection (exposure) and these diseases (outcome). On the other hand, the odds ratio for reflux esophagitis and sequelae is less than 1 which indicates the protective role of H pylori infection.

➢ Diagnostic laboratory tests

- Smears

  A gastroscopy procedure with biopsy is required. Routine stains demonstrate gastritis, and Giemsa or special silver stains can show the curved or spiral-shaped organisms.

- Culture

  [Culture is performed when patients are not responding to treatment, and there is a need to assess susceptibility patterns.]

- Special Tests
  - ✓ One of the quick tests is catalase reaction (they are catalase positive).
  - ✓ Rapid tests to detect urease activity in vitro are widely used for presumptive identification of H pylori in specimens.
  - ✓ **In vivo non-invasive tests** for urease activity can also be done (the most commonly used). In urea breath tests, 13 C-or 14 C-labeled urea is ingested by the patient. If H pylori is present, the urease activity

generates labeled CO2 that can be detected in the patient's exhaled breath.

- ✓ Detection of H pylori antigen in stool specimens is appropriate as a test of cure for patients with known H pylori infection who have been treated (to confirm complete eradication).

➢ Treatment

- ✓ Triple therapy with amoxicillin, clarithromycin and PPIs for 7-10 days, with a cure rate of more than 90%.
- ✓ There are some H pylori organisms that acquire resistance against erythromycin, thus a quadrable regimen is indicated which includes PPIs, metronidazole, amoxicillin and bismuth subsalycilate for 10 days.

Note: PPIs are given to induce ulcer healing, [they also appear to be potent urease inhibitors.]

❖plesiomonas

- Oxidase positive, gram negative rods with polar flagella.
- Uncommonly, plesiomonas are associated with diarrheal disease in humans.
- They are more common in tropical and subtropical areas.
- They live in fresh water, soil, and have been isolated from freshwater fish and many animals.
- They are resistant to O/129 compound which differentiates it from V. cholera.
- Plesiomonas species are positive for DNase; this and other biochemical tests distinguish it from Aeromonas species.
- [Some Plesiomonas strains share antigens with Shigella sonnei, and cross-reactions with Shigella antisera occur.]
- [Plesiomonas can be distinguished from shigellae in diarrheal stools by the oxidase test: Plesiomonas is oxidase positive, and shigellae are not.]
-

❖Aeromonas

- Aeromonas species are distinguished from the enteric gram-negative rods by finding a positive oxidase reaction in growth obtained from a blood agar plate.
- Aeromonas species are differentiated from vibrios by showing resistance to the compound O/129 and [lack of growth on media containing 6% NaCl.]

- [Typically, aeromonas produce hemolysins. Some strains produce an enterotoxin, Cytotoxins and the ability to invade cells in tissue culture have been noted.]
- [However, gastroenteritis, caused mostly by A caviae complex, ranges from acute watery diarrhea to less commonly a dysenteric type of illness.]
- [Aeromonas species are also associated with extraintestinal infections such as bacteremia and wound infections. The latter are often the result of trauma that occurs in a water environment and are caused primarily by A hydrophila.]

✓ Although plesiomonas and aeromonas are aquatic bacteria like vibrios, they can't be grown on the selective agar plates used to grow vibrios.
✓ Infection with plesiomonas and aeromonas ranges from mild gastroenteritis to wound infections to systemic infections especially in immunocompromised people.

➢ Treatment

Aeromonas and Plesiomonas are susceptible to fluoroquinolones (e.g. ciprofloxacin) and cephalosporins, [carbapenems and aminoglycosides], but they develop resistance very rapidly, because Aeromonas can produce β-lactamases, including carbapenemases, thus treatment should be guided by antibiotic susceptibility tests.

يا نفسُ صبراً فعُقبى الصّبر صالحةٌ .. لا بدّ أن يأتيَ الرحمنُ بالفرجِ ..فَصَبْرٌ جَمِيلٌ♡

Good Luck