Non- Fermentative Gram Negative Rods

Pseudomonas

Characteristics of *Pseudomonas*

- Gram-negative bacilli belonging to ***Pseudomonadaceae***
- Motile
- Capsulated "Polysaccharide capsule"
- Aerobic, Non fermentative, using carbohydrates through respiratory metabolism. Breakdown glucose by oxidation
- Oxidase positive (It is used to differentiate them from the *Enterobacterales)*
- Catalase positive

- very simple nutritional requirements - non fastidious
- The most important pathogenic organism is *P. aeruginosa*
- Optimum temperature is 37 C, and it is able to grow at 42 C
- resistant to high concentrations of salts, dyes, weak antiseptics, and many antibiotics
- Common inhabitants of soil, water, GIT

Pseudomonas aeruginosa

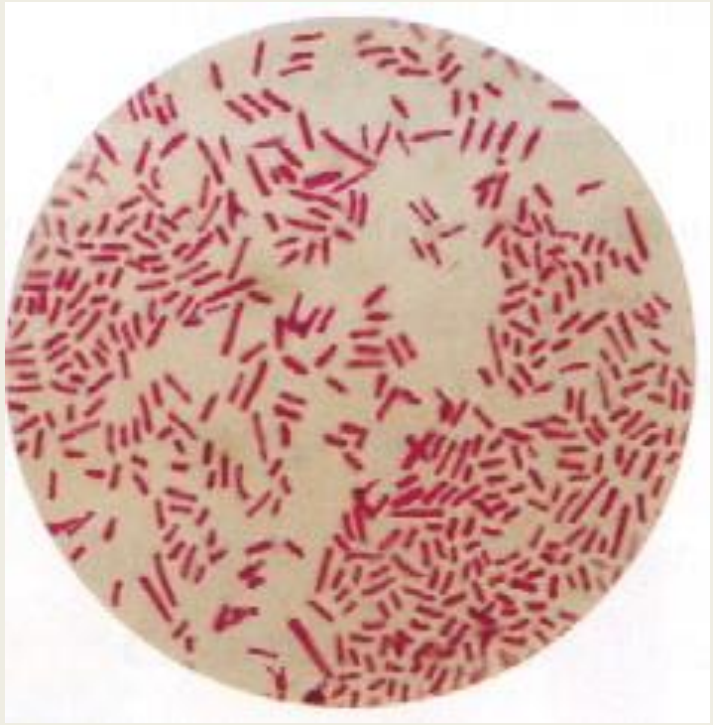
- Ubiquitous
  - *Soil, decaying organic matter, vegetation, water*
  - *Hospital environment*
- Moist reservoirs, food, cut flowers, sinks, toilets, floor mops, respiratory therapy & dialysis equipment
  - *EVEN "**disinfectant solutions**"*

Simple growth requirements

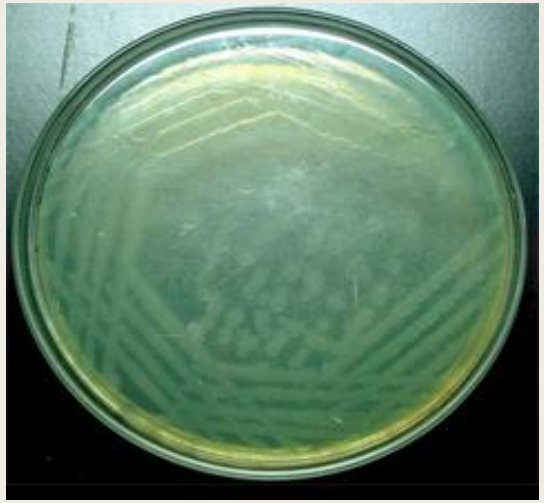
(can even grow in distilled water!!!!!!!)

- *P. aeruginosa* produce two types of soluble pigments:
  - ***Pyoverdin or fluorscein:*** *It is yellow-green pigment and fluorescent*
  - ***Pyocyanin:*** *It is a blue-green pigment and non-fluorescent*

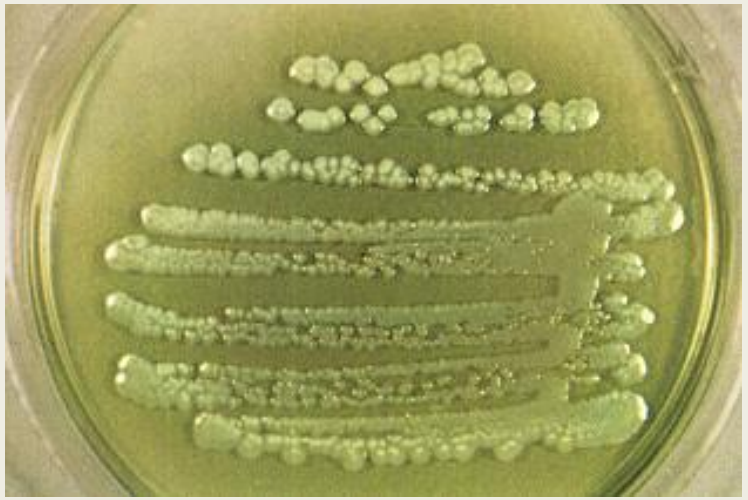
Cultural Characteristics



Gram Stain of *Pseudomonas*



P. aeruginosa on Cetrimide agar



P. aeruginosa on Nutrient agar

http://textbookofbacteriology.net/pseudomonas.html

Pseudomonas /Pathogenesis & Immunity

- Multiple virulence factors
  - *Structural components: adhesins (e.g., flagella, pili, LPS, alginate capsule)*
  - *Toxins & enzymes, pigments: exotoxin A, pyocyanin, pyoverdin, elastases, proteases, phospholipase C, exoenzymes S and T*
  - *Antibiotic resistance*

- **Adhesins**: Adherence of *P. aeruginosa* to host cells is mediated by pili and non-pilus adhesins.
- **Polysaccharide capsule**: also known as mucoid exopolysaccharide, **alginate** coat or glycocalyx. The capsule protects the organism from phagocytosis and activity of antibiotics.
- **Endotoxin**: Major cell wall antigen in *P. aeruginosa*, as it is in other gram negative rods.
- **Pyocyanin**: catalyzes the production of superoxide and hydrogen peroxide, toxic forms of oxygen.

- **Exotoxin A**: It is believed to be one of the most important virulence factor of pathogenic *P. aeruginosa*. This toxin disrupts protein synthesis by blocking peptide chain elongation in eukaryotic cell.
- **Exoenzymes S and T**: They are extracellular toxins produced by *P. aeruginosa*. When the type III secretion system introduces the proteins into their target eukaryotic cells, epithelial cell damage occurs, facilitating bacterial spread, tissue invasion and necrosis.
- **Elastases:** Two enzymes LasA (serine protease) and LasB (zinc metalloprotease) degrate elastin, resulting in damage to elastin-containing tissues and producing the lung paranchymal damage and hemorrhagic lessions (ecthyma gangrenosum).

- **Alkaline protease:** Contributes to tissue destruction and spread of *P. aeruginosa*. It also interferes with the host immune response.
- **Phospholipase C:** heat labile hemolysin that breaks down lipids and lecithin, facilitating tissue destruction.
- Antibiotic resistance: mutations of porin proteins, prevent penetration of antibiotics to the bacterial cell.

Virulence Factors Associated with *Pseudomonas aeruginosa*

Virulence Factors	Biologic Effects
Structural Components	
Capsule	Mucoid exopolysaccharide; adhesin; inhibits antibiotic (e.g., aminoglycoside) killing; suppresses neutrophil and lymphocyte activity
Pili	Adhesin
Lipopolysaccharide (LPS)	Endotoxin activity
Pyocyanin	Impairs ciliary function; stimulates inflammatory response; mediates tissue damage through production of toxic oxygen radicals (i.e., hydrogen peroxide, superoxide, hydroxyl radicals)
Toxins and Enzymes	
Exotoxin A	Inhibitor of protein synthesis; produces tissue damage (e.g., skin, cornea); immunosuppressive
Exotoxin S	Inhibits protein synthesis; immunosuppressive
Cytotoxin (leukocidin)	Cytotoxic for eukaryotic membranes (e.g., disrupts leukocyte function, produces pulmonary microvascular injury)
Elastase	Destruction of elastin-containing tissues (e.g., blood vessels, lung tissue, skin), collagen, immunoglobulins, and complement factors
Alkaline protease	Tissue destruction; inactivation of interferon and tumor necrosis factor-α
Phospholipase C	Heat-labile hemolysin; mediates tissue damage; stimulates inflammatory response
Rhamnolipid	Heat-stable hemolysin; disrupts lecithin-containing tissues; inhibits pulmonary ciliary activity
Antibiotic resistance	Complicates antimicrobial therapy

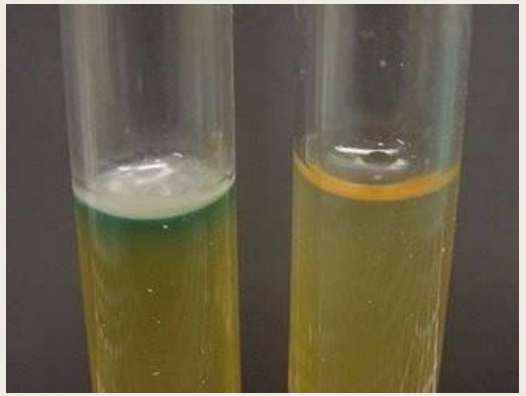
Pseudomonas / Clinical diseases

- Pulmonary infections (cystic fibrosis)
- Burn wound & other skin & soft tissue inf.
- UTI(Urinary tract infections)
- External otitis
- Eye infection (contaminated contact lens cleaning fluids)
- Bacteremia & endocarditis

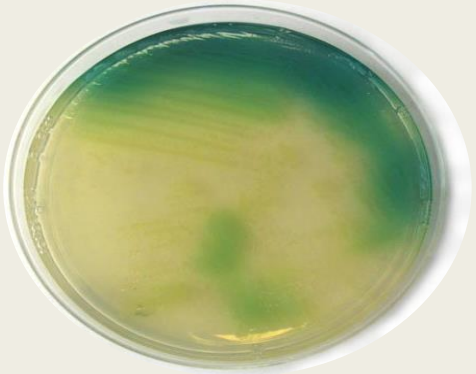
Pseudomonas / Diagnosis & Identification

- Culture
  - *Simple media*
- Identification
  - *Colonial morphology : colony size, hemolysis, pigmentation, odor*
  - *Biochemical tests: Positive oxidase test*

Pseudomonas /diagnosis & identification



- Culture:
  - *beta hemolysis,*
  - *green pigment,*
  - *grapelike odor*
  - *and simple biochemical tests (e.g., positive oxidase reaction)*



Pseudomonas /Treatment

- Combined use of effective antibiotics (e.g., aminoglycoside and β-lactam antibiotics) frequently required;
- Monotherapy is generally ineffective and can select for resistant strains

- Hospital infection-control efforts should concentrate on
  - *preventing contamination of sterile medical equipment and nosocomial transmission;*
  - *unnecessary use of broad-spectrum antibiotics can select for resistant organisms*

- *P. aeruginosa* also produces a number of different β-lactamases
- That can inactivate many β-lactam antibiotics (e.g., penicillins, cepha losporins, and carbapenems): treatment problem because of limited antibiotic choice

KEY POINTS FOR PSEUDOMONAS

- Pseudomonas are aerobic, innately resistant bacteria causing opportunistic infections in human. Pseudomonas regularly isolated from human infections.
- *P. aeruginosa* is frequently isolated and causes infections that may be trivial and life threatening.
- Mucoid form of *P. aeruginosa* is a major cause of chronic debilitating and life-threatening respiratory infections in individuals with cystic fibrosis.

Burkholderia

This group consists

- *Burkholderia mallei,*
- *Burkholderia pseudomallei,*
- *Burkholderia pickettii,*
- *Burkholderia cepacia*

Burkholderia mallei

- *B. mallei* is a small, gram-negative, oxidase-positive, non-encapsulated, aerobic bacillus. Unlike *B. pseudomallei,* **it is nonmotile.** It is a host-adapted pathogen that, unlike *B. pseudomallei,* does not persist in the environment outside its equine host. The *Burkholderia* genome projects and multi- locus sequence typing have supported the idea that *B. mallei* evolved in animals from the environmental pathogen *B. pseudomallei.*

- When It leave from the organism, growth in the general medium becomes difficult. The presence of glycerin, egg yolk in the medium makes it easier to growth. Optimal temperature is 37° C and pH 7.6 . It can not grow at 4 ° C. it is not very active as biochemically. It is not resistant to heat, dryness and antiseptic. It can not form extracellular and intracellular pigment and exotoxin.

Burkholderia mallei

- It is a pyogenic bacterium. It's endotoxin is poisonous. Veterinarians, animal carers, farmers and laboratory workers may be affected by this disease.
- Glanders is a highly communicable disease of solipeds (horses, donkeys, and mules) that is caused by *Burkholderia mallei.* It can be transmitted to other animals and to humans.

- In acute form, general condition disorder, joint pain, fever are observed. First, vesicles form in the nasal mucosa. Mucous, irritable excretion occurs Then pus swelling occur in the skin and muscle. Lymph nodes are also swollen and inflamed. Untreated animals die in 2- 4 weeks. Untreated people die in 10 days. There is no long-term immunity at the end of the disease.

Burkholderia pseudomallei

- *B. pseudomallei* is a small, gram-negative, oxidase-positive, motile, aerobic bacillus with occasional polar flagella. On staining, a bipolar "**safety pin**" pattern is seen. The organism is easily recovered on standard culture medium.

- The organism is present in soil and surface water in endemic regions. It is common in Philippines, South East Asia, North Australia, Papua New Guinea.
- The lesion is the characteristic of the disease. The agent mainly enters through the skin and spreads by hematogenous pathway (lung, liver, spleen, bone, skin, etc.)

Image copyright Dennis Kunkel Microscopy
http://www.denniskunkel.com/

The infections of *B. pseudomallei*

- Lung infection
- Acute suppurative infection.
- Chronic suppurative infection.
- Septicemic **melioidosis** (95% fatal) 50% success with treatment
- Effective: Tetracycline, clopramenicol, TMP-SMZ
- Ineffective: Ampicillin, erythromycin, gentamycin, streptomycin, penicillin

Burkholderia cepacia

- Burkholderia cepacia complex (BCC), is a group of catalase-producing, lactose-nonfermenting, Gram-negative bacteria composed of at least 18 different species, including *B. cepacia*, *B. multivorans, B. cenocepacia, B. vietnamiensis, B. stabilis, B. ambifaria, B. dolosa, B. anthina, B. pyrrocinia and B. ubonensis*

- *B. cepacia* is an opportunistic human pathogen that most often causes pneumonia in immunocompromised individuals with underlying lung disease (such as cystic fibrosis or chronic granulomatous disease. mortality rate is given as 35% in pneumonia.

- It has been found topical antiseptic betadine and recently in mouthwash.
- Treatment includes ceftazidime, doxycycline, piperacillin, meropenem, chloramphenicol and trimethoprim / sulfamethoxazole.
- The first option is TMP / SMZ,

- However, when hypersensitivity, intolerance or resistance occur, other alternatives are chosen.
- It is naturally resistant to aminoglycosides and polymyxin B. They are used for selecting from other species by adding to the medium.

KEY POINTS FOR BURKHOLDERIA

- *Burkholderia mallei* is an agent of **Glanders** that is a highly communicable disease of solipeds.
- *Burkholderia pseud*omallei is the causative agent of **melioidosis**, a systemic infection of human and animals in South-East Asia and northern Australia.
- The *Burkholderia cepacia* complex causes life-threatening pulmonary infections in individulas with cystic fibrosis or chronic granulomatous disease.

Vibrio

- *Vibrio cholerae,*
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*

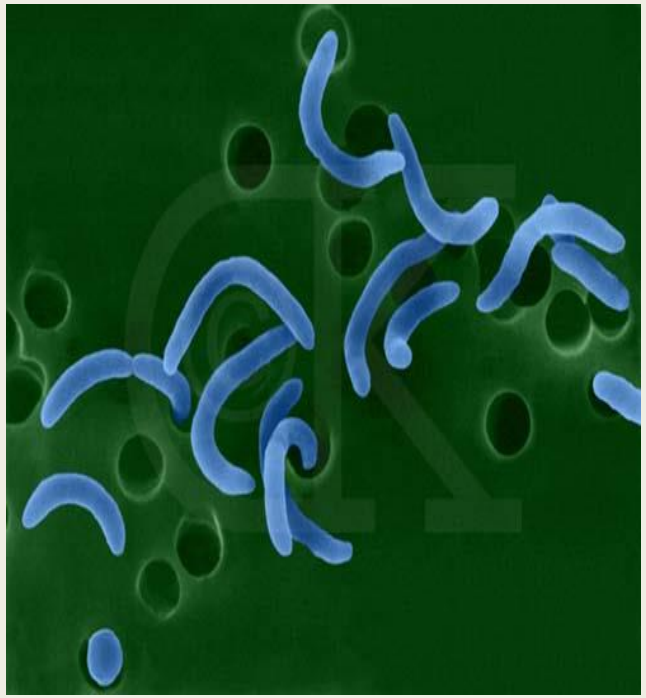
The General Characteristic of Vibrio

- Gram negative
- Slightly convoluted, hard-bodied
- They are very motile with one or more flagella
- Oxidase(+), Gelatinase (+), Catalase (+), Indole (+).
- They never form urease and H_2S.

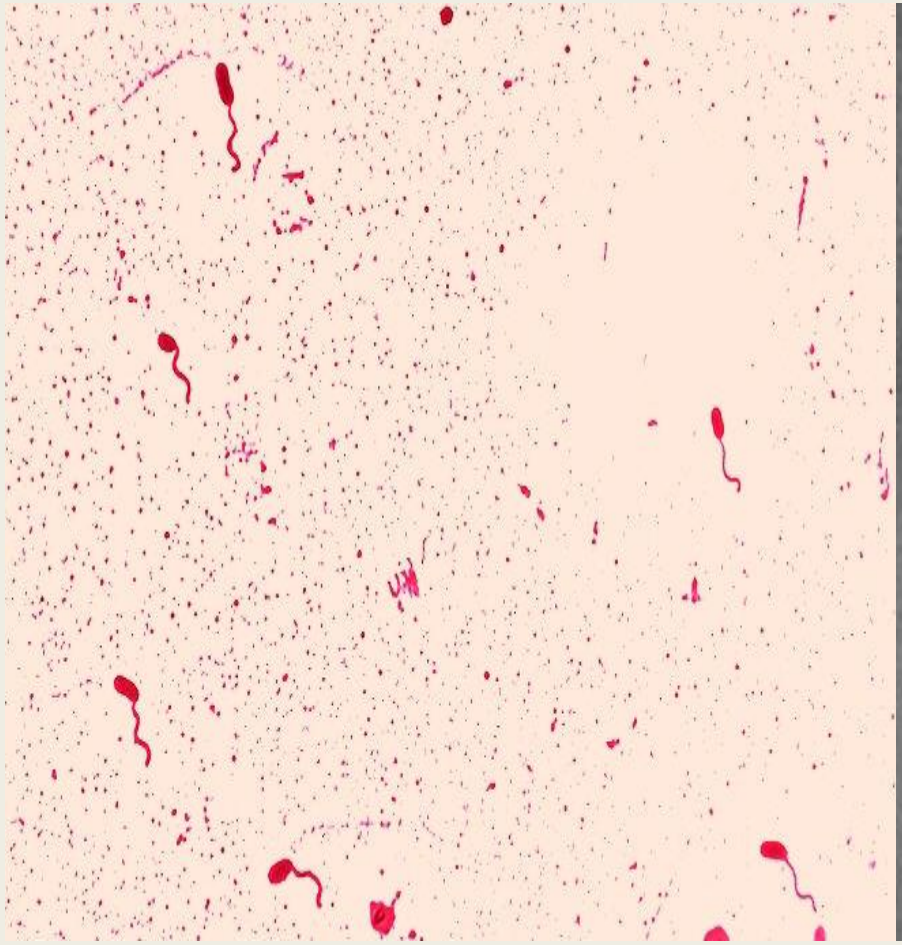
- Can grow on a variety of simple media within a broad spectrum temperature range (14- 40 ^{o}C). *V. cholerae* can grow in the absence of salt, most other species that are pathogenic in humans require salt.
- Vibrios tolerate a wide range of pH but are susceptible to stomach acids. If gastric acid production is reduced or neutralized, patients are more susceptible to Vibrio infections.

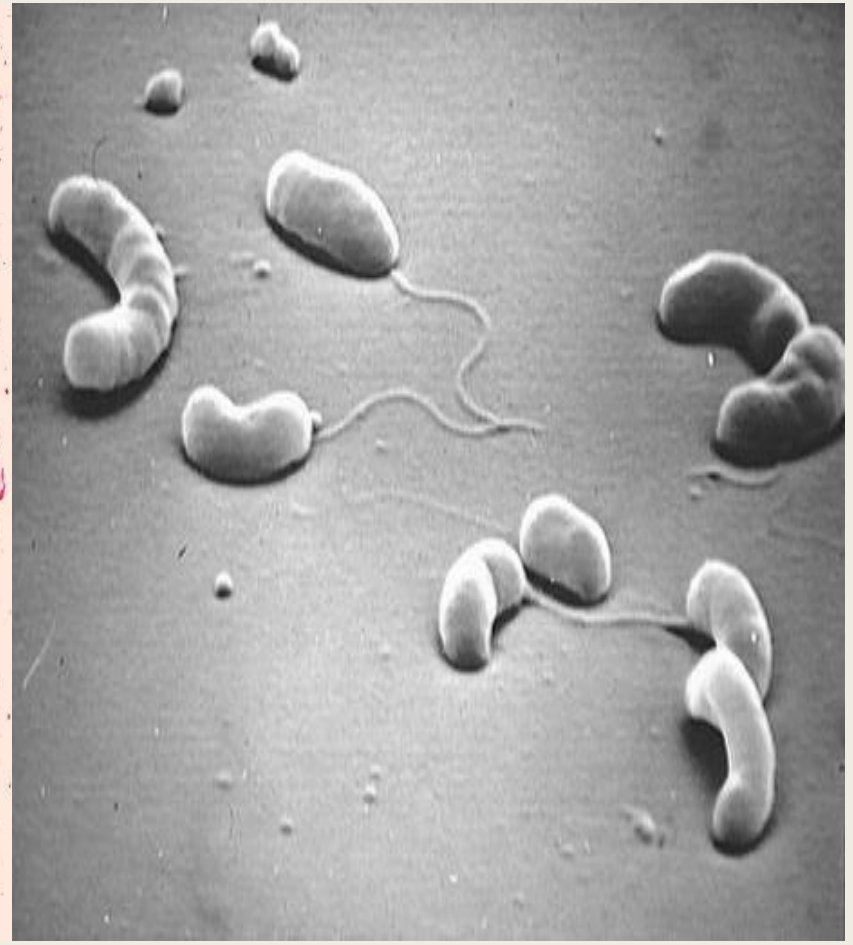
Vibrio cholerae

- It is a rod similar to commas. A few can be seen as S, C together.
- Involution and spheroplast forms can be in old culture.
- very motile with flagella.
- growth easy in alcali medium.
- weak against acid
- melt gelatin and coagulant serum
- Have musinase and RDE(receptor destroying enzyme)
- Give firstly colorless and then pink colony in MacConkey



Vibrio cholerae's light and electron microscopy images





Antigenic structure

- H antigen
- LPS (O) antigen
- Three major subgroups;
- 1. *V. cholerae* O1
  2. *V. cholerae* O139
  3. *V. cholerae* non-O1 (O2-O138)
- *V. cholerae* O1 and O139 Bengal produce cholera toxin and associated with epidemics of cholera.

- *V. cholerae* O1 is further subdivided into serotypes and biotypes. Three serotypes are recognized:
  - Inaba
  - Ogawa
  - Hikojima

- Two biotypes of *V. cholerae* O1 recognized:
  - Classical (First six pandemic)
  - Eltor (seventh pandemic)

The differences between Classical and eltor biotypes

Features	Classical	Eltor
Hemolysis on sheep blood agar	-	+
Agglutination of chicken erythrocytes	-	+
CAMP test	-	+
Voges-Proskauer	-	+
Polymyxin B susceptibility	+	-

Resistance

- Sensitive to heat, disinfectant, dryness and acids
- 15 min at 55 ° C. also die.
- in 5% phenol. also die in 2 minutes.
- Sensitive against most of the chemotherapeutics (chloramphenicol, tetracycline, gentamycin, ampicillin)

Virulence

- Motility
- Pilus
- Hemaglutinin
- Musinase
- Capsule
- Cholera toxin

Epidemiology f Cholera

- Major pandemics of cholera have occurred since 1817 resulting in thousands of deaths. Sporadic disease and epidemics occured before this time but worldwide spread of the disease became possible with intercontinental travel.
- The seventh pandemic which is caused by *V. cholerae* 01 biotype eltor in Asia in 1961 and spread to Africa, Europe in 1970s, 1980s. In 1991 the pandemic strain spread to Peru.
- *V. cholerae* 0139 Bengal emerged in 1992 in India

The mechanism of action of cholera toxin

- Bacteria attach to upper intestine via pili and subsequently colonize

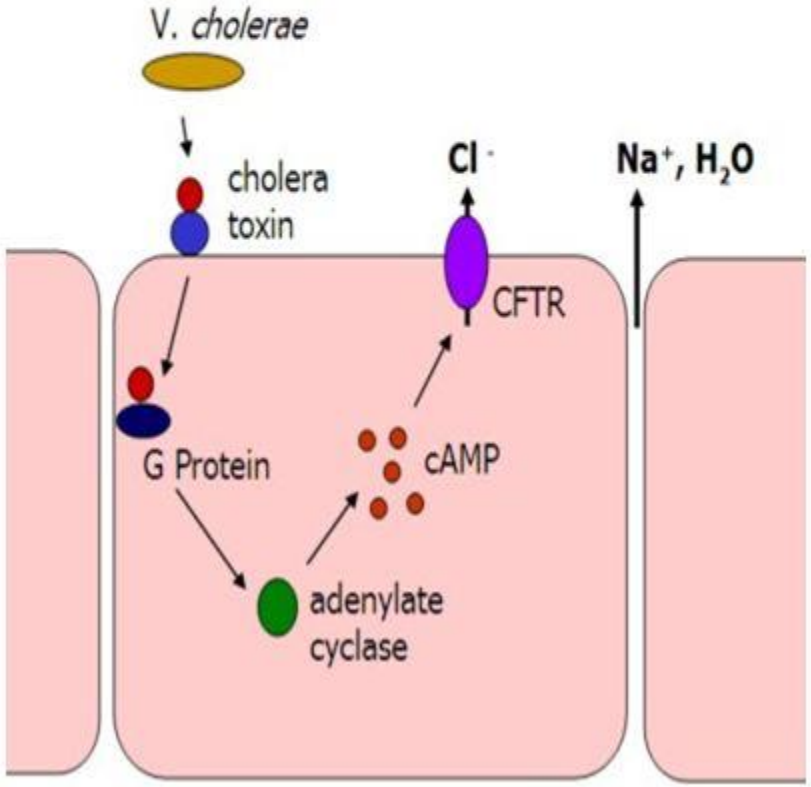
Cholera enterotoxin

- *1 A subunit*
- *5 B subunits*

- B subunits bind to a GM1 ganglioside receptor on the mucosal cell to allow entry of the A subunit
- A subunit Overactivates Adenylate Cyclase (Gs protein)
- Hypersecretion of Water, Chloride and other

Cholerae toxin

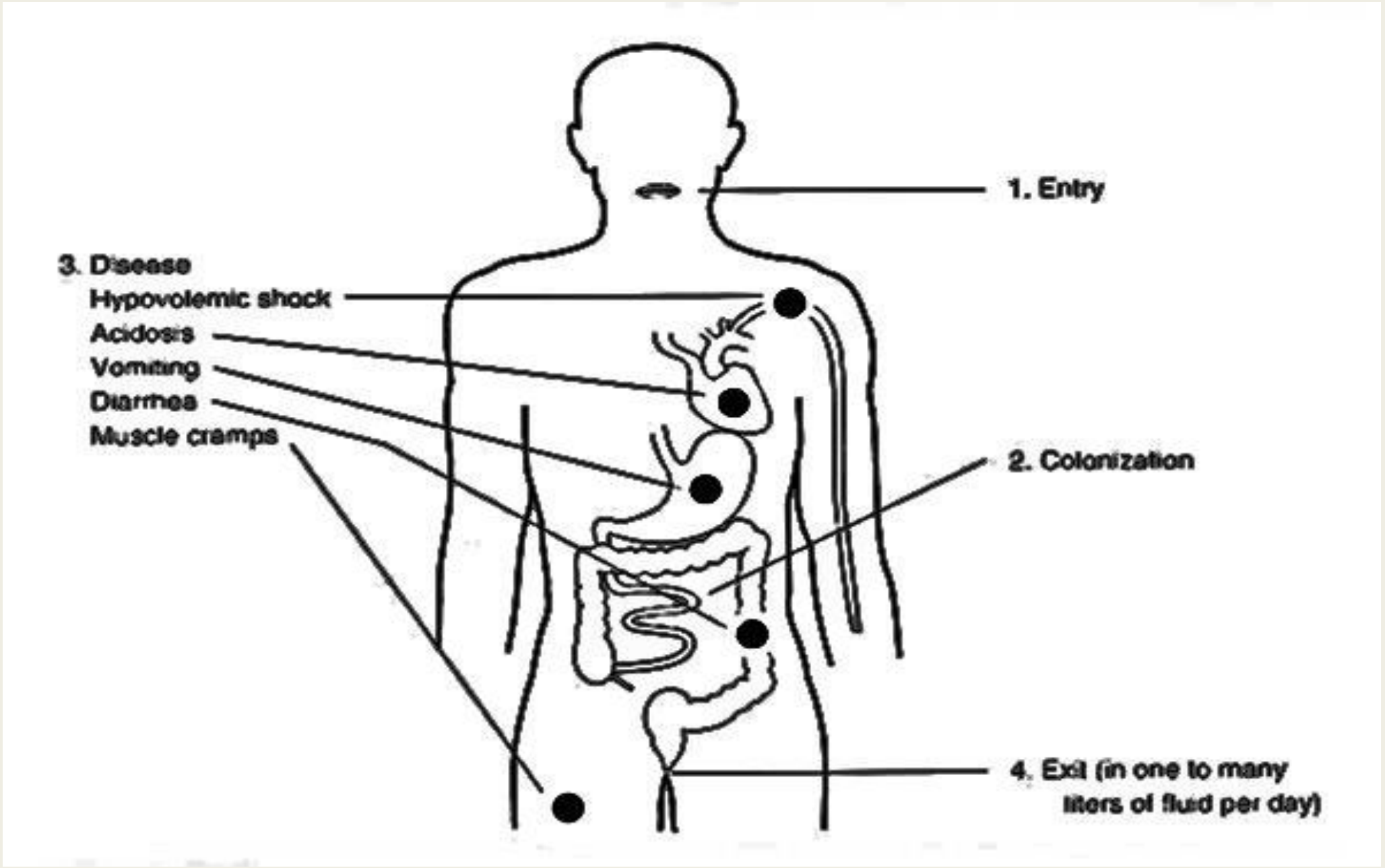
- Cholera toxin is released from bacteria in the gut lumen and binds via the B subunit to GM1 receptors on enterocytes, triggering endocytosis.
- The A subunit enzymatically activates a G protein and locks it into its GTP-bound form through an ADP-ribosylation reaction.
- G protein activity leads to activation of adenylyl cyclase and increased cAMP levels.
- High cAMP levels then go on to activate the membrane-bound CFTR protein, leading to dramatic efflux of chloride, sodium, and water from the intestinal epithelium.



Clinic of cholera

- The incubation period is 2-3 days.
- The disease starts with sudden vomiting and diarrhea.
- The feces is first fecaloid, then the rice water look and fishy smell.
- In 5-12 hours;
- Urine is diminished, it can be an anuria.

- There is no fever, but cyanosis. (Cyanosis is defined as the bluish or purplish discolouration of the skin or mucous membranes due to the tissues near the skin surface having low oxygen saturation.)
- There are painful cramps in the arms and legs.
- Apati, **hands like launderer**
- Scaphoid abdomen but is soft. The reflexes are diminished.
- As the disease progresses, heart sounds are weak.
- With acidosis and coma, death occurs in a few days.


1. Entry
3. Disease
Hypovolemic shock
Acidosis
Vomiting
Diarrhea
Muscle cramps
2. Colonization
4. Exit (in one to many liters of fluid per day)

Cholerae Disease

- The northern hemisphere is most visible in summer and autumn.
- In endemic societies, 10 times more from adults are seen in the 1-5 age group. Every age is in equal risk for new serotype cases.

1. Asymptomatic

2. Cholera diarrhea

3. Slightly cholera (cholerin)

4. Cholera gravis (mainly cholera type)

5. Cholera sikka (it is the heaviest form.) There is death with collapse and shock without the opportunity to defecate.

6. Typhoid cholera (seen in more children with fever at 38-39 ° C.)

Diagnosis

- Vibrio can remain alive for 14 days in room temperature in peptone water and 21 days in plastic bag method.
- 1d From Fresh stool or 4-6 hours peptone water cultivation + 1d. O group serum is match. Aggl.
- The preparation prepared from the material is examined by fluorescent antibody technique. It is not used in the first instance.
- Serological diagnosis with two serially serums is useful for follow-up.

Vibrio parahaemolyticus

- The first was found by Fujino in food poisoning caused by eating of seafood in Japan.
- Halophilous
- Side flagella may also form additionally to polar flagella.
- It growths in simple medium at 2 % salt concentrations. It’s growth increase by 7-8 % salt concentrations.

- Generation time: 9-15 minutes. Lactose (-) .
- The disease is caused by toxins, which are **thermolabile hemolysin** of the vibrios that settle in the gut and multiply in the mucosa.
- It is seen with severe vomiting and abundant watery diarrhea 6-20 hours after food intake. It usually heals in 2-3 days.
- Diagnosis: from the stool.

V. vulnificus

- It is a particularly virulent species of Vibrio responsible for rapidly progressive **wound infections** after exposure to contaminated seawater and **septicemia** after consumption of contaminated raw **oysters**.
- The antimicrobial therapy is important especially immunocompromised patients.

Treatment, prevention and control of Vibrio

- Patients with cholera must be promptly treated with fluid and electrolyte replacement.
- Antibiotic therapy, although of secondary value, can reduce toxin production and more rapidly eliminate the organism. Doxycycline or tetracyline is the drug of choice for adults.
- *V. parahaemolyticus* gastroenteritis is usually a self limited disease, although antibiotic therapy can be used in addition to fluid and electrolyte therapy in patients with severe infection.
- V. vulnifucus wound infections and septicemia must be promptly treated with antibiotic.

KEY POINTS FOR VIBRIO

- *V. cholerae* belonging to serogroup O1 and O139 are the agent of epidemic cholera.
- Cholera toxin is the key pathogenic mechanism, causing extensive loss of water and electrolytes in the form of rice-water stools; death from cholera can be prevented with rehydration therapy.
- All Vibrio types need salt but *V. cholerae* can growth without salt

- *V. parahaemoltyicus* is a major cause of diarrhea in South-East Assia (infection is associated with the consumption of seafood.
- Infection with *V. vulnificus* may result in rapid onset and fatal septicemia, particularly in people with conditions of iron overload, and is associated with the consumption of seafood. *V. vulnifucus* cause wound infection

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