



Gram-Negative Rods

Vibrio disease

Lecture topics:-

A- *Vibrio cholerae* .

General Features ✓

Clinical signs ✓

Laboratory identification ✓

Treatment ✓

B- *Vibrio parahaemolyticus*

C- *Vibrio vulnificus*

References.



Pathogen Within the Enteric Tract

Vibrio disease:-

A- *Vibrio cholerae*

General Features:

- 1- Gram negative rods curve or comma shaped, non-spore forming, highly motile single polar flagellum (polar monotrichous) .
- 2-Associated with surface water, salt waters (e.g. rivers, lakes, coastal waters). facultative anaerobe , halophilic .
- 3-*V.cholerae* is transmitted by fecal contamination of water and food, the main animal reservoirs are marine shellfish such as(oysters) and fish. Ingestion of these without adequate cooking can transmit the disease.



4- **Pathogenesis**; They contain lipopolysaccharide (LPS) in their cell wall, which is both antigenic and a potential **virulence factor** (endotoxin). In addition, several exotoxins are producing by *V. cholerae*, these exotoxins are called **enterotoxines** causing diarrhea. The two surface antigens :-

- ❑ Cell wall antigen or somatic Ag (O Ag).
- ❑ The flagellar antigen (H Ag).

5- Antigenic structure common heat labile flagellar **H antigen**, **O** (somatic) lipopolysaccharide, serogroup, **O1**, two biotypes (Classical biotype, El Tor biotype) and **O139** cause **epidemic** and **pandemic** cholera .

Clinical signs :-

1- Asymptomatic colonization (60% with classic and 75% with El Tor). Incubation period 1-4 days.

2- Abrupt onset of nausea, **watery diarrhea** and vomiting. **Rice water stools** (contains mucus, epithelia cells and vibrio's).

3- Results in dehydration, hypovolemic shock, renal failure (anuria), cardiac, coma and death, mortality rates 25- 50 % if untreated.



B- Vibrio parahaemolyticus

Is a marine organism transmitted by ingestion of raw or undercooked seafood especially shellfish such as oysters. **Halophilic** vibrio. cause **food poisoning** in human beings' ingestion of raw seafood. Symptoms bloody diarrhea, headache, abdominal cramps, nausea, vomiting and fever. can also cause wound infections in people exposed to contaminated seawater. No specific treatment is indicated because the disease is relatively mild and **self-limited**.

C-Vibrio vulnificus

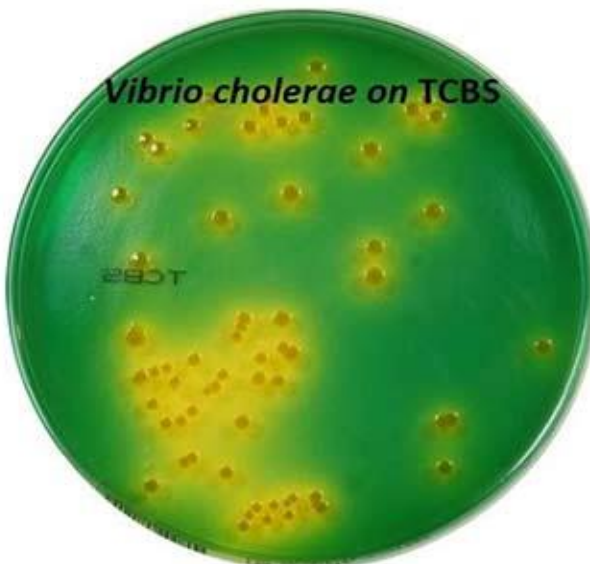
Is also a marine organism (it is found in warm salt waters ,common oysters). It causes severe wound infections, bacteremia, and gastroenteritis. It causes wound infection (cellulitis, myositis and necrosis), bacteremia after ingestion mainly in people with liver disease (cirrhosis), 50% of patient with bacteremia die.

The medically important vibrios

| Organism | Human Disease |
|---|---|
| <i>Vibrio cholerae</i> serogroups O1 and O139 | Epidemic and pandemic cholera |
| <i>Vibrio cholerae</i> serogroups non-O1/non-O139 | Cholera-like diarrhea; mild diarrhea; rarely, extraintestinal infection |
| <i>Vibrio parahaemolyticus</i> | Gastroenteritis, wound infections, septicemia |
| <i>Vibrio vulnificus</i> | Gastroenteritis, wound infections, septicemia |

Laboratory identification:-

1- Specimens from to **stools**, smear not perfect but dark –field microscopy or phase –contrast may reveal vibrio’s, culture *V. cholerae* grows MacConkey agars. Thiosulfate citrate bile salts sucrose (TCBS) medium can enhance isolation. Vibrio cholera produce (yellow colonies) while in *Vibrio vulnificus* & *Vibrio parahaemolyticus* produces green colonies on TCBS.



Vibrio cholerae on TCBS Agar



Vibrio parahaemolyticus on TCBS Agar



Vibrio cholerae colony morphology on Macconkey medium non lactose fermenter colonies of Vibrio (form normal-colored (i.e., un-dyed) colonies (Lactose negative) . While if lactose positive lactose fermentation bacteria results **pink colonies**.

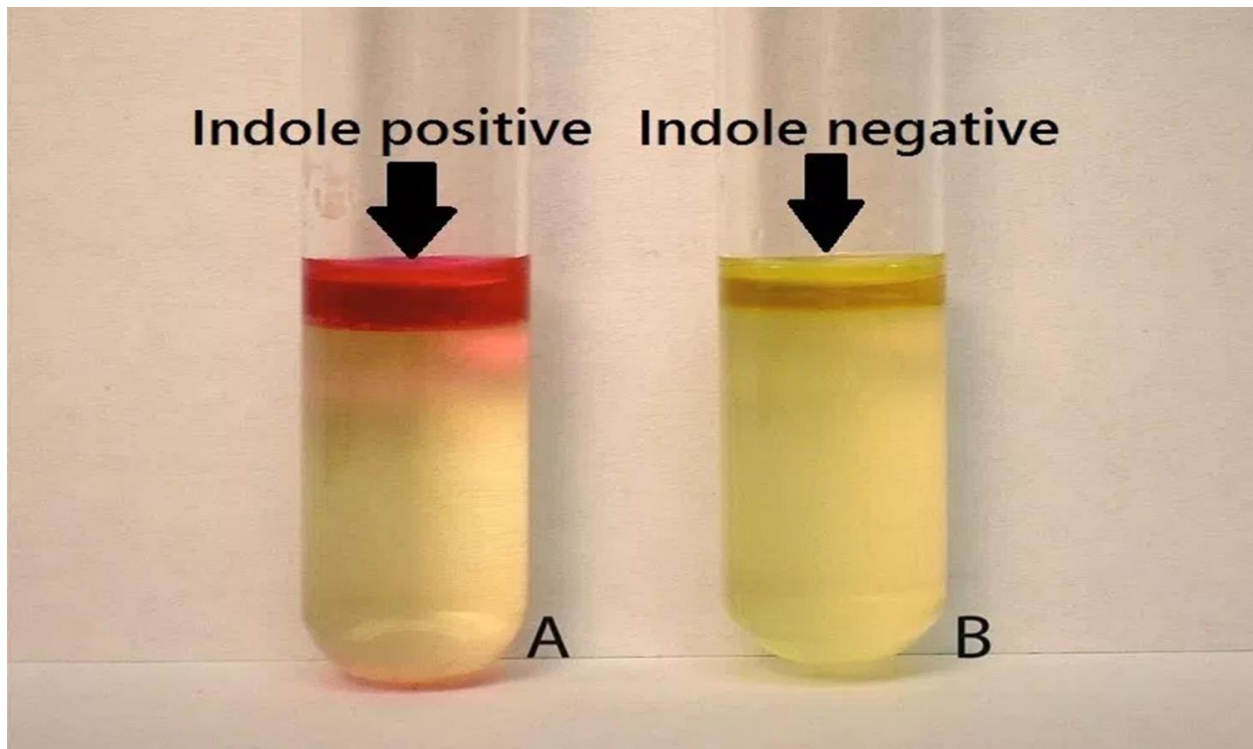


2- Catalase , Oxidase , Simmon's Citrate Agar , Indole :- Positive.

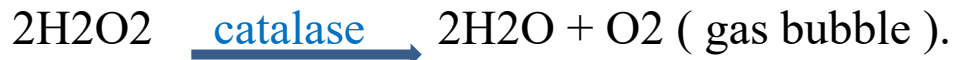
- ✓ **Indole Medium Test** :- determines the ability of an organism to produce indole from the breakdown of the amino acid **tryptophan** .Tryptophan is hydrolyzed by **tryptophanase enzyme** present in **bacteria** to produce indole. Indole production is demonstrated by the addition of **Kovac's reagent** which acts with the indole giving a **red color**. The main requirement for a suitable indole test medium is that it contains a sufficient amount of **tryptophan**. (Tryptophan broth, peptone broth, Sulfide-indole motility medium (SIM). Inoculate the tryptophan (or peptone) broth with the organism to be tested and incubate at 37 ° C for 24 to 48 hours. Add 0.5 ml (5 drops) of **Kovác's reagent**.

Then formation of **red color** ring at top of broth indicated a **positive reaction** while a **yellow color** ring indicated a **negative reaction**.

Tryptophan $\xrightarrow{\text{tryptophanase}}$ Indole + Kovac's reagent = red color ring.

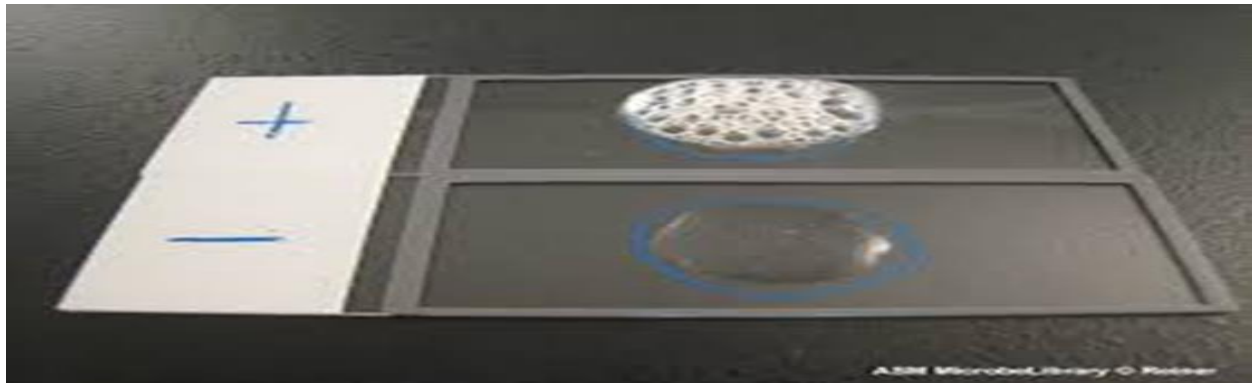


- ✓ **Catalase test** :- the enzyme catalase mediates the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water. The presence of the enzymes in bacterial isolated is evident when a small inoculum is introduced into hydrogen peroxide and rapid elaboration oxygen (bubbles) and water.

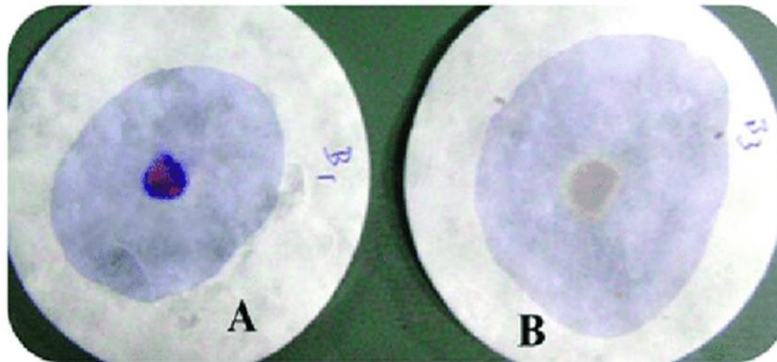


The result \longrightarrow positive test.

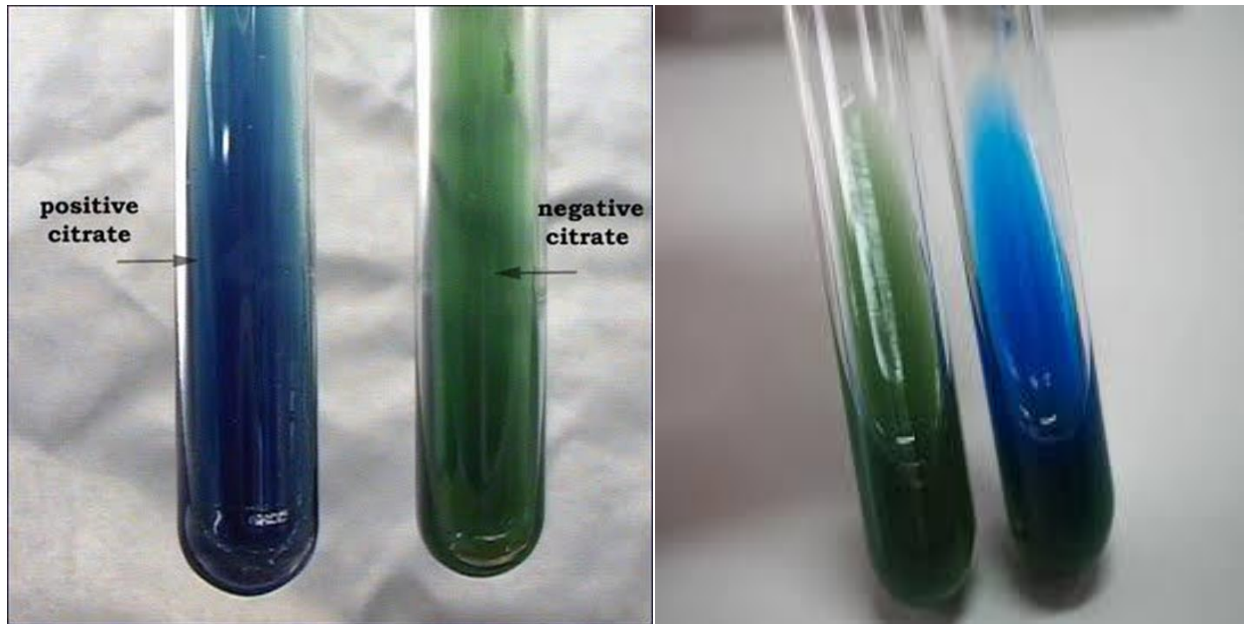
No bubbles result \longrightarrow negative test.



- ✓ **Oxidase Test** :- Used to determine if a bacterium produce of **cytochrome c oxidase**. small area of filter paper is soaked with a freshly prepared 1% oxidase reagent , bacterial colony to be tested is picked from agar by stick or glass rod and put it on the soaked area. **(A) positive result** is indicated by formation of deep **purple color** (dark blue) while the reagent is colorless when the **(B) negative result**.



- ✓ **Simmon's Citrate Agar Test** :- is an agar medium used for the differentiation of Enterobacteriaceae based on the utilization of citrate as the sole source of carbon. In organisms capable of using **citrate** as a carbon source, the enzyme citrase hydrolyzes citrate into **oxaloacetic acid** and acetic acid. The oxaloacetic acid is then hydrolyzed into **pyruvic acid** and **CO₂**. If CO₂ is produced, it reacts with the components of the medium to produce an alkaline compound (eg Na₂CO₃). The alkaline pH changes the pH indicator (bromthymol blue) from **green** to **blue**.



3- Slide agglutination with anti O group 1or 139 antisera .

The use of antisera is one of the most rapid and specific methods of identifying *V. cholerae*. Agglutination tests for *V. cholerae* somatic O antigens may be carried out in a petri dish or on a clean glass slide. Mix the suspension and antiserum well and then tilt slide back and forth to observe for agglutination. If the reaction is positive, very strong clumping will appear within 30 seconds to 1 minute.



Confirmatory Tests for V.cholerae

- V.cholerae organisms are further identified by slide agglutination tests using anti-O group 1 or group 139 Antisera and by Biochemical reactions



4-Triple Sugar Iron (TSI):- test is a microbiological test roughly named for its ability to test a microorganism's ability to **ferment sugars** and to produce **hydrogen sulfide (black color)**. It is often used to differentiate enteric bacteria including *Salmonella* and *Shigella*.

TSI agar

Triple Sugar Iron Agar

(a) Red/red (no sugar fermentation)

(b) Control

(c) Red/yellow (Glucose fermented but lactose and sucrose not fermented)

(d) Yellow/yellow (Glucose fermented. Lactose and/or sucrose fermented)

(e) Red/yellow with H₂S

A B C D E




Figure 5-68

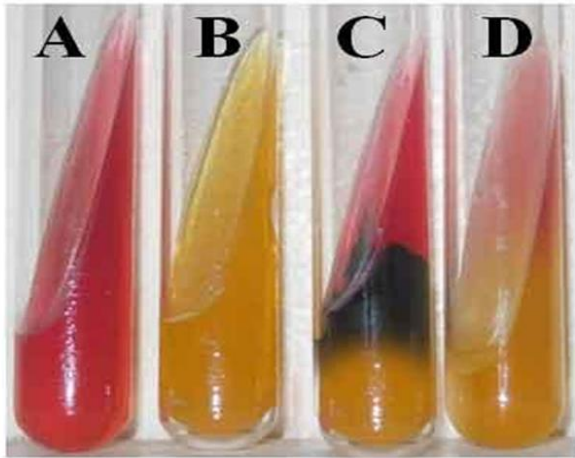
0.1% dextrose

1.0% sucrose

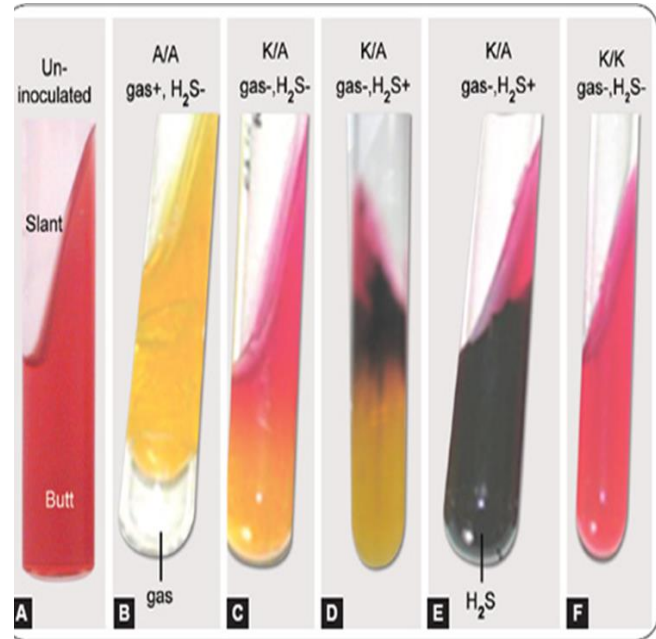
1.0% lactose

Interpretation of Triple Sugar Iron Agar Test:-

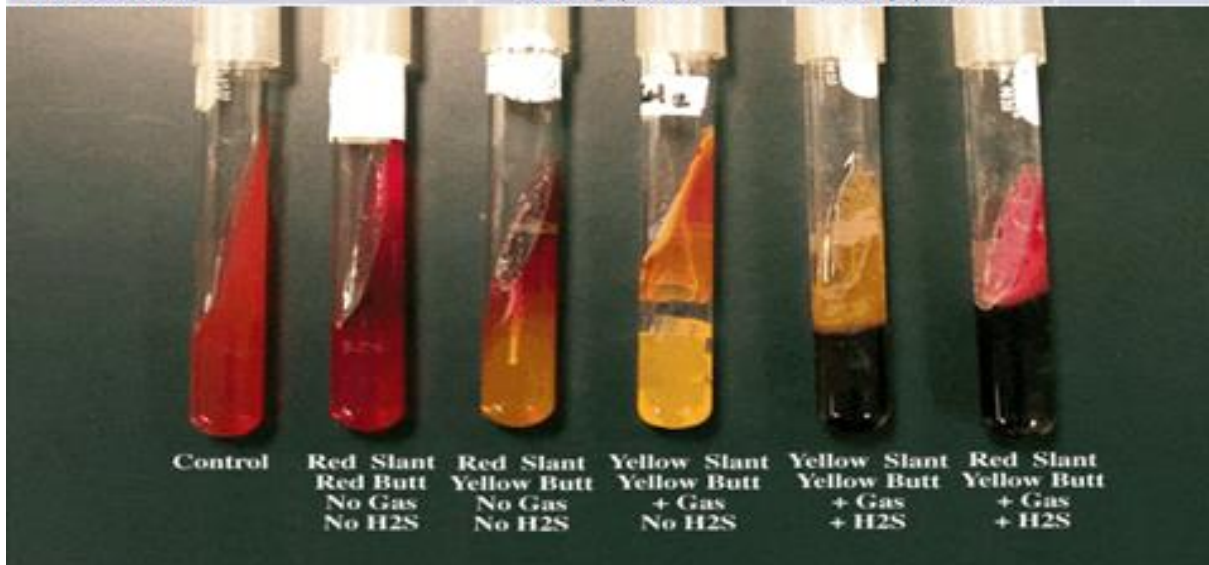
- Alkaline slant/Alkaline butt (K/K) i.e. Red/Red = glucose, lactose and sucrose non-fermenter.
- Alkaline slant/acidic butt (K/A); Red/Yellow = glucose fermentation only, gas (+ or -), H₂S (+ or -).
- Acidic slant/acidic butt (A/A); Yellow/Yellow = glucose, lactose and/or sucrose fermenter gas (+ or -), H₂S (+or-).



- A) *Psuedomonas aeruginosa*: Gluc (-), Lac/Suc (-), H₂S (-)
- B) *Escherichia coli*: Gluc (+), Lac/Suc (+), H₂S (-)
- C) *Salmonella typhimurium*: Gluc (+), Lac/Suc (-), H₂S (+)
- D) *Shigella boydii*: Gluc (+), Lac/Suc (-), H₂S (-)



| Bacteria | Slant | Butt | Gas | H ₂ S |
|---|------------------|------------------|-----|------------------|
| <i>E.coli</i> , <i>Klebsiella</i> , <i>Enterobacter</i> | Acid (A) yellow | Acid (A) yellow | + | - |
| <i>Shigella</i> , <i>Serratia</i> . | Alkaline (K) red | Acid (A) yellow | - | - |
| <i>Salmonella</i> , <i>Proteus</i> . | Alkaline (K) red | Acid (A) yellow | + | + |
| <i>Pseudomonas</i> . | Alkaline (K) red | Alkaline (K) red | - | - |
| <i>Vibrio cholera</i> | Acid (A) yellow | Acid (A) yellow | - | - |



Treatment:-

The basic, overall treatment for Cholera is re-hydration, to replace the fluids that have been lost. Those with mild dehydration can be treated orally with an oral rehydration solution (ORS). When patients are severely dehydrated and unable to take in the proper amount of ORS, IV fluid treatment is generally pursued. Antibiotics are used in some cases, typically fluoroquinolones and tetracycline's.

References

1. Thompson FL, Gevers D, Thompson CC, et al. (2005). ["Phylogeny and Molecular Identification of Vibrios on the Basis of Multilocus Sequence Analysis"](#). *Applied and Environmental Microbiology*. **71** (9): 5107–5115. [Bibcode:2005ApEnM..71.5107T](#). [doi:10.1128/AEM.71.9.5107-5115.2005](#). [PMC 1214639](#). [PMID 16151093](#).
2. [^] Ryan KJ; Ray CG, eds. (2004). *Sherris Medical Microbiology (4th ed.)*. McGraw Hill. [ISBN 978-0-8385-8529-0](#).
3. [^] [Jump up to:^{a b}](#) Faruque SM; Nair GB, eds. (2008). ["Vibrio cholerae: Genomics and Molecular Biology"](#). Caister Academic Press. [ISBN 978-1-904455-33-2](#).
4. [^] [Jump up to:^{a b c d}](#) Paul, Sulav Indra; Rahman, Md. Mahbubur; Salam, Mohammad Abdus; et al. (2021-12-15). ["Identification of marine sponge-associated bacteria of the Saint Martin's island of the Bay of Bengal emphasizing on the prevention of motile Aeromonas septicemia in Labeo rohita"](#). *Aquaculture*. **545**: 737156. [doi:10.1016/j.aquaculture.2021.737156](#). [ISSN 0044-8486](#).
5. [^] Madigan, Michael; Martinko, John, eds. (2005). *Brock Biology of Microorganisms (11th ed.)*. Prentice Hall. [ISBN 978-0-13-144329-7](#).
6. [^] Khan, Fazlurrahman; Tabassum, Nazia; Anand, Raksha; Kim, Young-Mog (2020-10-01). ["Motility of Vibrio spp.: regulation and controlling strategies"](#). *Applied Microbiology and Biotechnology*. **104** (19): 8187–8208. [doi:10.1007/s00253-020-10794-7](#). [ISSN 1432-0614](#). [PMID 32816086](#). [S2CID 221182959](#).



7. ["Laboratory Methods for the Diagnosis of *Vibrio cholerae*"](#) (PDF). Centre for Disease Control. Retrieved 29 October 2013.
8. ["What is *Vibrio parahaemolyticus*?"](#), EHA Consulting Group, retrieved 25 September 2018
9. ^ Jump up to:^{a b} Ryan KJ, Ray CG, eds. (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. [ISBN 0-8385-8529-9](#).
10. ^ Finkelstein RA (1996). ["Cholera, *Vibrio cholerae* O1 and O139, and Other Pathogenic Vibrios."](#) In Baron S, et al. (eds.). *Barron's Medical Microbiology* (4th ed.). University of Texas Medical Branch at Galveston. [ISBN 0-9631172-1-1](#) – via NCBI Bookshelf).
11. ^ Baffone W, Casaroli A, Campana R, Citterio B, Vittoria E, Pierfelici L, et al. (2005). ["In vivo' studies on the pathophysiological mechanism of *Vibrio parahaemolyticus* TDH\(+\)-induced secretion"](#). *Microbial Pathogenesis*. **38** (2–3): 133–137. [doi:10.1016/j.micpath.2004.11.001](#). [hdl:11576/2504278](#). [PMID 15748815](#).
12. Reichelt JL, Baumann P, Baumann L (October 1976). ["Study of genetic relationships among marine species of the genera *Beneckea* and *Photobacterium* by means of in vitro DNA/DNA hybridization"](#). *Arch. Microbiol*. **110** (1): 101–20. [doi:10.1007/bf00416975](#). [PMID 1015934](#). [S2CID 23759213](#).
13. ^ Jump up to:^{a b} Farmer JJ (October 1979). ["Vibrio \("*Beneckea*"\) *vulnificus*, the bacterium associated with sepsis, septicaemia, and the sea"](#). *Lancet*. **314** (8148): 903. [doi:10.1016/S0140-6736\(79\)92715-6](#). [PMID 90993](#). [S2CID 34979437](#).
14. ^ Jump up to:^{a b} Oliver JD (2005). ["Wound infections caused by *Vibrio vulnificus* and other marine bacteria"](#). *Epidemiol Infect*. **133** (3): 383–91. [doi:10.1017/S0950268805003894](#). [PMC 2870261](#). [PMID 15962544](#).
15. ^ ["Glowing" Seafood?"](#) (PDF). U.S. Food And Drug Administration Seafood Products Research Center. [Archived](#) (PDF) from the original on 2020-11-27. Retrieved 2021-03-09.
16. ^ James, William D.; Berger, Timothy G. (2006). *Andrews' Diseases of the Skin: Clinical Dermatology*. Saunders Elsevier. [ISBN 978-0-7216-2921-6](#).
17. ^ Jump up to:^{a b c} Hollis DG, Weaver RE, Baker CN, Thornsberry C (April 1976). ["Halophilic *Vibrio* species isolated from blood cultures"](#). *J. Clin. Microbiol*. **3** (4): 425–31. [doi:10.1128/jcm.3.4.425-431.1976](#). [PMC 274318](#). [PMID 1262454](#).
18. ["Vibrio Species Causing Vibriosis"](#). Centers for Disease Control. [Archived](#) from the original on June 16, 2017. Retrieved June 5, 2017.
19. ["UpToDate"](#). [www.uptodate.com](#). [Archived](#) from the original on 2019-06-21. Retrieved 2019-06-21.
20. ["Vibrio Species Causing Vibriosis - Questions and Answers"](#). Centers for Disease Control. [Archived](#) from the original on June 7, 2017. Retrieved June 5, 2017.