Assist. Prof. Dr. Ameer Mezher Hadi
Dr.Zaid Abdel Hadi Abd
Lecture. 7
Second Stage

College of Health & Medical Techniques
Department of Medical Laboratories Techniques

VIBRIO

Introduction:

Vibrios are among the most common bacteria in marine and estuarine waters, worldwide. **They are comma-shaped, curved, and sometimes straight facultatively anaerobic, fermentative rods**; **they are catalase and oxidase positive**, and most species are motile **by means of monotrichous or multitrichous polar flagella**. Vibrios can grow within a broad temperature range **(14–40°C)**, and all species require **sodium chloride (NaCl)** for growth; hence the term **halophilic ("salt loving")**. ***V. cholerae* serogroups O1 and O139 cause cholera in humans**, and other vibrios, most commonly *V. parahaemolyticus* and *V. vulnificus*, are important human pathogens, causing skin and soft tissue infections, sepsis, or gastroenteritis.

VIBRIO CHOLERAE

The bacterium *V. cholerae* is the cause of cholera. The epidemiology of cholera closely parallels the recognition of ***V. cholerae* transmission in water and the development of sanitary water systems.** Cholera is associated with poor sanitation, as well as direct contact with or consumption of **contaminated water and/or food (eg, water used for drinking, cooking, bathing, and crop irrigation).**

Morphology and Identification

A. Typical Organisms

Upon first isolation, ***V. cholerae* is a comma-shaped, curved rod** 2–4 μm long (Figure-1). It is actively motile by means of a **polar flagellum.** On prolonged cultivation, organisms may become straight rods that can resemble other Gram negative enteric bacteria.

B. Culture

V. cholerae **produces convex, smooth, round colonies that are opaque and granular in transmitted light.** *V. cholerae* and most other vibrios grow well at 37°C on routine agar media to recover enteric bacteria **(eg, blood agar and MacConkey agar)**; however, selective agars for *Vibrio* species, such as **thiosulfate-citrate-bile salts-sucrose (TCBS) agar** and enrichment broth **(eg, alkaline peptone broth)**, can also be used to recover vibrios, especially from specimens **(eg, stool)** when a mixture of organisms is expected.

All vibrios, including *V. cholerae*, grow well on TCBS agar; *V. cholera* produces yellow **colonies (sucrose fermented) on TCBS agar that are readily visible against the dark-green background of the agar** (Figure-2). Non-sucrose-fermenting vibrios (eg, most strains of *V. parahaemolyticus* and *V. vulnificus*) produce green colonies on TCBS agar. Characteristically, **vibrios grow at a very high pH (8.5–9.5)** and are rapidly killed by acid. To ensure optimal recovery of vibrios, stool specimens should be collected early in the course of the diarrheal illness; prompt inoculation onto appropriate agar media is necessary. If processing of specimens may be delayed, the stool specimen should be mixed in a Cary-Blair transport medium and refrigerated.

In areas where cholera is endemic, direct cultures of stool on selective media, such as TCBS, and enrichment broth cultures (eg, alkaline peptone water with 1% NaCl, pH 8.5) are appropriate. In the United States and other countries where cholera is rare, routine use of TCBS agar for stool cultures in clinical laboratories is generally not necessary or cost effective; exceptions may be made if recovery of other vibrios (eg, *V. parahaemolyticus*) is a frequent and/or seasonal occurrence (eg, coastal U.S. regions with regular and frequent consumption of bivalve mollusks and crustaceans).

Table-1: The Medically Important Vibrios

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

C. Growth Characteristics

V. cholerae regularly ferments sucrose and mannose but not arabinose. A positive oxidase test result is a key step in the preliminary identification of *V. cholerae* and other vibrios. While most *Vibrio* species are halophilic, requiring the presence of NaCl (range from < 0.5–4.5%) to grow, *V. cholerae* can grow on most agar media without additional salt.

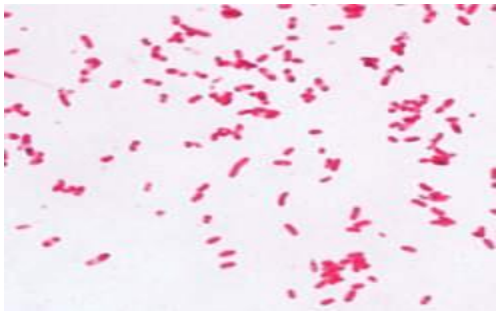

Figure-1: Gram-stain of *V. cholerae*. Often they are comma shaped or slightly curved (arrows) and 1 × 2 to 4 µm. Original magnification ×1000

VIBRIO

Figure-2: Colonies of *V. cholerae* growing on thiosulfate, citrate, bile salts, and sucrose agar. The glistening yellow colonies are 2–3 mm in diameter and are surrounded by a diffuse yellowing of the indicator in the agar up to 1 cm in diameter. The plate is 10 cm in diameter.

Antigenic Structure and Biologic Classification

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.

V. cholerae has O lipopolysaccharides that confer serologic specificity. Based on the O antigen, there are over 200 serogroups; however, only *V. cholerae* strains of serogroup O1 and O139 cause epidemic and pandemic cholera. Occasionally, non-O1/non-O139 *V. cholerae* strains have been described as causes of cholera-like diarrheal disease. Antibodies to the O antigens tend to protect laboratory animals against infections with *V. cholerae*.

The *V. cholerae* serogroup O1 antigen has determinants that make possible further subtyping; these serotypes are **Ogawa, Inaba, and Hikojima**. Furthermore, **two biotypes of epidemic *V. cholerae* have been defined, classic and El** Tor. **The El Tor biotype produces a hemolysin**, gives positive results on the Voges-Proskauer test, and is resistant to polymyxin B. Molecular techniques can also be used to type *V. cholerae*. Typing is

used for epidemiologic studies, and tests generally are done only in reference laboratories. *V. cholerae* O139 is very similar to *V. cholerae* O1 El Tor biotype. *V. cholerae* O139 does not produce the O1 lipopolysaccharide and does not have all the genes necessary to make this antigen. *V. cholerae* O139 and other non-O1 *V. cholerae* strains, as well as *V. vulnificus* produce acidic polysaccharide capsules; however, *V. cholerae* O1 does not make a capsule.

***Vibrio cholerae* Enterotoxin**

V. cholerae produce a heat-labile enterotoxin with a molecular weight (MW) of about 84,000, consisting of subunits A (MW, 28,000) and B (MW, 56,000).

Ganglioside GM1 serves as the mucosal receptor for subunit B, which promotes entry of subunit A into the cell. Activation of subunit A 1 yields increased levels of intracellular cyclic adenosine monophosphate (cAMP) and results in prolonged hypersecretion of water and electrolytes. There is increased sodium-dependent chloride secretion, and absorption of sodium and chloride by the microvilli is inhibited. Electrolyte-rich diarrhea occurs with as much as 20–30 L/day, resulting in dehydration, shock, acidosis, and death. The genes for *V. cholerae* enterotoxin are located on the bacterial chromosome. **Cholera enterotoxin is antigenically related to LT of *Escherichia coli* and can stimulate the production of neutralizing antibodies**. However, the precise role of antitoxic and antibacterial antibodies in protection against cholera is not clear.

Diagnostic Laboratory Tests

A. Specimens

As stated above, **stool specimens should be collected early in the course of the diarrheal** illness and inoculated within 2–4 hours of collection onto appropriate agar media, to ensure

optimal recovery of vibrios. If processing of specimens may be delayed, the stool specimen should be mixed in a Cary-Blair transport medium and refrigerated.

B. Smears

Direct detection of *V. cholerae* on smears made from stool samples is not distinctive of the organism, and therefore not routinely recommended. Dark-field or phase-contrast microscopy can be used to detect *V. cholerae* O1 directly from stool samples or the enrichment broth. Observation of "shooting star" motility is suggestive of *V. cholerae* O1; if the motility is extinguished after mixing the sample with a polyvalent O1 antiserum, the organism is confirmed as *V. cholerae* O1. However, if there is no motility or the type of motility does not change after applying the antiserum, the organism is not *V. cholerae* O1.

C. Culture

Vibrios, including *V. cholerae*, grow well on most agar media (including MacConkey and blood agar) used in clinical laboratories. Some strains of *V. cholerae* may however be inhibited on MacConkey agar. **Growth is rapid in alkaline peptone broth or water, containing 1% NaCl with a pH of 8.5, or on TCBS agar; typical colonies can be picked in 18 hours of growth. For enrichment, a few drops of stool can be incubated for 6–8 hours in taurocholate peptone broth (pH, 8.0–9.0);** organisms from this culture can then be stained or subcultured onto other appropriate agar media. Accurate identification of vibrios, including *V. cholerae*, using commercial systems and kit assays is quite variable. **MALDI-TOF MS is a promising newer methodology for identification of vibrios, and studies have shown rapid and reproducibly accurate identification for *V. parahaemolyticus***.