



VIBRIO AND RELATED ORGANISM

27.1 INTRODUCTION

Vibrios are Gram-negative, rigid, curved rods that are actively motile by means of a polar flagellum. The name 'Vibrio' is derived from the characteristic vibratory motility (from vibrare, meaning to vibrate). They are asporogenous and noncapsulated. Vibrios are present in marine environments and surface waters worldwide. The most important member of the genus is *Vibrio cholerae*, the causative agent of cholera. It was first isolated by Koch (1883) from cholera patients in Egypt, though it has been observed earlier by Pacini (1884) and others.



Fig. 27.1: Vibrio Cholera



OBJECTIVES

After reading this lesson, you will be able to:

- describe the salient characteristics of *Vibrio cholerae*.

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- describe the pathogenesis, clinical presentation, diagnosis, management and prevention of cholera.
- list the features of other pathogenic vibrios, such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*.

27.2 VIBRIO CHOLERAЕ

Morphology : The cholera vibrio is a short, curved, cylindrical rod, about $1.5 \times 0.2-0.4$ mm in size, with rounded or slightly pointed ends. The cell is typically comma shaped (hence the old name V comma) but the curvature is often lost on subculture. S-shaped or spiral form may be seen due to two or more cells lying end to end. Pleomorphism is frequent in old cultures. In stained films of mucus flakes from acute cholera cases, the vibrios are seen arranged in parallel rows, described by Koch as the 'fish in stream' appearance. It is actively motile, with a single sheathed polar flagellum. The motility is of the darting type, and when acute cholera stool or a young culture is examined under the microscope, the actively motile vibrios suggest a 'swarm of gnats'. The vibrios stain readily with aniline dyes and are Gram negative and non-acid fast.

Culture Characteristics

The cholera vibrio is strongly aerobic, growth being scanty and slow anaerobically. It grows within a temperature range of 16-40°C (optimum 37 °C), Growth is better in an alkaline medium, the range of pH being 6.4-9.6 (optimum 8.2). NaCl (0.5-1%) is required for optimal growth though high concentration (6% and above) are inhibitory.

It grows well on ordinary media. On MacConkey's agar, the colonies are colourless at first but become reddish on prolonged incubation due to the late fermentation of lactose. On blood agar, colonies are initially surrounded by a zone of greening, which later become clear due to hemodigestion. In peptone water, growth occurs in about six hours as a fine surface pellicle, which on shaking breaks up into membranous pieces.

A number of special media have been employed for the cultivation of cholera vibrios. They may be classified as follows :

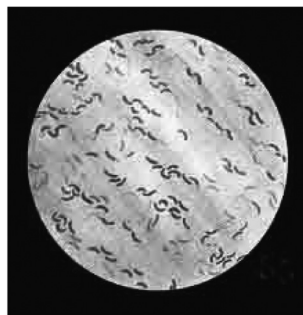


Fig. 27.2: Microscopic Slide of Vibrio Cholera

MODULE

Microbiology



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Holding or transport media

1. Venkatraman – Ramakrishnan (VR) medium : In this medium vibrios do not multiply but remain viable for several weeks.
2. Cary-Blair medium : It is a suitable transport medium for Salmonella and Shigella as well as for vibrios.

Enrichment media

1. Alkaline peptone water at a pH of 8.6;
2. Monsur's taurocholate tellurite peptone water at pH 9.2.

Both these are good transport as well as enrichment media.

Plating media

1. Alkaline bile salt agar (BSA) at pH 8.2 : The colonies after overnight growth, colonies are moist, translucent, round discs, about 1-2mm in diameter; with a bluish tinge in transmitted light.
2. TCBS medium : This medium, containing thiosulfate, citrate, bile salts and sucrose, is available commercially and is very widely used at present. Cholera vibrios produce large yellow convex colonies which may become green on continued incubation.

Biochemical reactions : Carbohydrate metabolism is fermentative, producing acids, but no gas. Cholera vibrios ferment glucose, mannitol, maltose, mannose and sucrose but not inositol, arabinose or lactose, though lactose may be split very slowly. Indole is formed and nitrates are reduced to nitrites. These two properties contribute to the 'cholera red reaction' which is tested by adding a few drops of concentrated sulphuric acid to a 24-hour peptone water culture. With cholera vibrios, a reddish pink colour develops due to the formation of nitroso-indole, Catalase and oxidase testes are positive. Methyl red and urease tests are negative. Vibrios decarboxylate lysine and ornithine but not utilize arginine.

Resistance : Cholera vibrios are susceptible to heat, drying and acids, but resist high alkalinity. They are destroyed at 55°C in 15 minutes. Dried on linen or thread, they survive for 1-3 days but die in about three hours on cover slips. Survival in water is influenced by its pH temperature, salinity, presence of organic pollution and other facts. In general, the E1 Tor vibrio survives longer than the classical cholera vibrio. In the laboratory, vibrios survive for months in sterile sea water, and this has been suggested as a method for the survival of vibrios in nature. They survive in clean tap water for thirty days. In untreated night soil, they may survive for several days. Vibrios are susceptible to common disinfectants.

On fruits, they survive for 1-5 days at room temperature and for a week in the refrigerator. In general, food materials left at room temperature do not act as an important source of infection for longer than a day or two but those stored in the cold may harbour vibrios for more than two weeks.

They are killed in a few minutes in the gastric juice of normal acidity but they may survive for 24 hours in achlorhydric gastric juice.

Classification

1. Serological Classification
 2. According to Growth requirement
1. Serological Classification : VIBRIO are classified according to biochemical properties & H antigen into Group A and Group B.
 - (i) Group A : This group includes vibrio cholerae and have similar biochemical properties and Common H (flagellar) Antigen. Group A is again classified based on somatic O antigen into O1 & Non-O1 O1 (agglutinable vibrio): These vibrios agglutinate with O1 antiserum.
 - (ii) Non-O1 (non-agglutinable vibrio) : This are not agglutinated by O1 antiserum. They are currently upto O-139.

O1 are classified into Biotypes-CLASSICAL and ELTOR and these are further classified into serotypes- OGAWA, INABA, HIKOJIMA

Subgroup O1 can further be classified into two groups as follows

	Classical	Eltor
Hemolysis	–	+
Voges Prosker test	–	+
Chick erythrocyte agglutination	–	+
Polymyxin B Sensitivity	+	–
Group IV phage susceptibility	+	–
Group V phage susceptibility	–	+

2. According to growth requirement
- Genus Vibrio includes 33 species the important are :

- 1.1 Non halophillic : It includes following & they are able to grow in media without salts.

V. cholerae

**Notes**

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Notes

1.2 Halophilic : They are unable to grow in media without salts – have a high requirement of NaCl.

V. Parahemolyticus is responsible for food poisoning

V. alginolyticus is responsible for diarrheal disease

V. vulnificus is responsible for diarrheal disease



INTEXT QUESTIONS 27.1

1. What is the causative agent of Cholera ?
2. Vibrio shows which type of motility ?
3. Which is the widely used medium for Cholera ?
4. Group A Vibrios has which common antigen ?
5. What is the requirement halophilic species ?

27.3 PATHOGENECITY OF VIBRIO CHOLERAEE

Cholera

Disease caused by *Vibrio Cholerae*

Introduction

Cholera is an acute diarrheal disease caused by *V. cholerae*. In its most severe form, cholera is a dramatic and terrifying illness in which profuse painless watery diarrhea and copious effortless vomiting may lead to hypovolemic shock and death in less than 24 hours. In treated cases, the disease may last 4-6 days, during which period the patient may pass a total volume a liquid stool equal to twice his body weight.

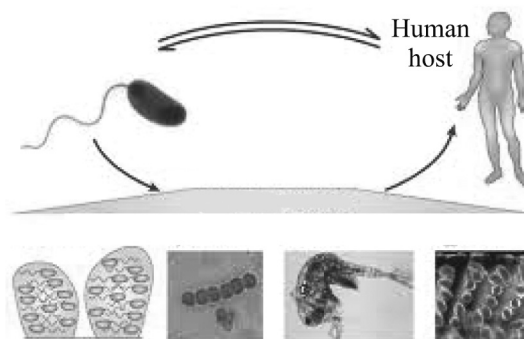


Fig. 27.3: Pathogenesis Disease

Clinical Feature of Cholera

- All the clinical features of severe cholera result from this massive loss of fluid and electrolytes.
- The cholera stool is typically a colourless watery fluid with flecks of mucus, said to resemble water in which rice has been washed (hence called '**rice water stools**').
- The clinical severity of cholera varies widely, from the rapidly fatal disease to a transient asymptomatic colonization of the intestine by the vibrios.
- The incidence of mild and asymptomatic infections is more with EI Tor vibrios than with the classical cholera vibrios.
- The incubation period varies from less than 24 hours to about five days. The clinical illness may begin slowly with mild diarrhea and vomiting in 1-3 days or abruptly with sudden massive diarrhea.

Pathogenesis

- Vibrio Cholerae enters through orally contaminated food & water.
- If gastric acidity is less then they will be destroyed otherwise they passes to intestine attaches the mucus membrane breach the epithelial cell & attach to produce toxin.

Cholera Toxin

- It is very similarly to heat labile toxin of E.coli in structural, biochemical, biological & antigenic properties but is more potent.
- It's production is determined by filamentous phage integrated with bacterial chromosome.

Mode of action

- Cholera Toxin has 2 sub units Sub-Unit A and Sub-Unit B
- Sub-Unit B binds to GM1 ganglioside on gut epithelial cells.
- This binding activates A subunit & it divides into A1 & A2.
- A1 activates adenly cyclase wich converts ATP to CAMP.
- CAMP increases the outflow of electrolytes & water to gut lumen & causes diarrhea.
- Sub-Unit A2 act as binding agent for A1 to B



Notes

27.4 LABORATORY DIAGNOSIS

Aim

- To demonstrate *Vibrio Cholerae* & identify the species.

Specimen**1. Stool**

- Collected in acute stage of the disease before administration of Antibiotics.
- Collected by introducing lubricated catheter into rectum or directly in screw capped bottle.

2. Rectal Swab

- Very useful in convalescent carrier in which there is no watery diarrhea.
- Transport Media for Cholera are as follows If chances of delay occur then use V-R medium, Bile peptone transport medium, Monsur's medium, Carry Blair medium, Autoclaved sea water

Methods

The Methods for demonstration of the disease are as follows:

1. Direct Demonstration of bacilli

- 1.1 Microscopy :For rapid diagnosis , the characteristic darting motility of vibrios and its inhibition by antiserum can be demonstrated hanging drop method using cholera stool from acute cases or more reliably after enrichment for 6 hours.

3. Culture

- Media

Enrichment media – Alkaline peptone water

For the plating medias are – Bile Salt Agar, & TCBS



Fig. 27.4: Colonies of V Cholerae on TCBS



Notes

- Method

If the laboratory facilities are available then the specimen which is collected is directly inoculated on plating media otherwise it is transported by holding media then inoculated on enrichment media for 6-8 hrs. then subculture on plating media after colonies appear the slide agglutination test with O1 antiserum is done after the species is biochemically identified.

4. Biochemical Reactions

- V Cholera is identified from other by Biochemical reaction as follows it shows Oxidate positive, Catalase Positive, Indole Positive, V.P. Positive test & Citrate Positive but it shows Urease & Methyl red test negative.

5. For isolation of carries

- Essentially the same technique is required only more than one cycle of enrichment is required.
- As the excretion of bacilli is intermittent repeated stool examination is required.

Prophylaxis

The prevention of cholera requires essentially general measures such as provision of protected water supply and improvement of environmental sanitation. An ideal cholera vaccine is yet to be found.

Other Vibrio

- Vibrio Mimicus

So named because it closely resembles cholera vibrios in biochemical features, V mimicus can be differentiated by its failure to ferment sucrose.

- Halophilic Vibrios

Vibrios that have a high requirement of sodium chloride are known as halophilic vibrios.

- Vibrio Parahaemolyticus

V parahaemolyticus is an enteropathogenic halophilic vibrio. It is the causative agent of an outbreak of food poisoning due to sea fish. Gastroenteritis due to this vibrio has since been identified in several countries and it is now considered an important cause of food poisoning throughout the world. It resembles the cholera vibrio, except that it is capsulated, shows bipolar staining and has a tendency to pleomorphism, especially when grown on 3% salt agar and in old cultures. It grows only in media containing NaCl.

**Notes**

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- **Vibrio Alginolyticus**

This halophilic vibrio resembles *V. parahaemolyticus* in many respects and was formerly considered a biotype of the latter. It has a higher salt tolerance, is VP positive and ferments sucrose.

- **Vibrio Vulnificus**

V. vulnificus, previously known as L+ vibrio or *Beneckea vulnifica*, is a marine vibrio of medical importance. It is VP negative and ferments lactose but not sucrose. It has a salt tolerance of less than 8 per cent. It causes two types of illness. The first is wound infection following contact of open wounds with sea water. The second type occurs in compromised hosts particularly those with liver disease. Following ingestion of the vibrio, usually in oysters, it penetrates the gut mucosa without causing gastrointestinal manifestations and enters the bloodstream, rapidly leading to septicemia with high mortality.

- **Aeromonas and Plesiomonas**

Aeromonas hydrophila, originally isolated from frogs, in which it causes the 'red leg disease', has been reported from many cases of diarrhea and from some pyogenic lesions in human beings. *Plesiomonas shigelloides* also has been reported from diarrheal disease.



INTEXT QUESTIONS 27.2

1. How long is the incubation period of *V. Cholerae* ?
2. *V. Cholerae* toxin is similar to which toxin ?
3. Toxin activates which enzyme in Gut Lumen ?
4. Before administration of antibiotics which specimen is collected for laboratory diagnosis ?
5. In Convalescent carrier which specimen is commonly collection ?
6. Which is the most common plating media for *Vibrio* ?



WHAT YOU HAVE LEARNT

- Members of the genus *Vibrio* are curved, gram negative rod-shaped bacteria which exhibit darting motility, are facultative anaerobes, positive by the catalase and oxidase tests. *Vibrios* are found in aquatic environments. In this genus, *Vibrio Cholerae* is the most important cause of human disease,

but *Vibrio parahaemolyticus* and *Vibrio vulnificus* are also sometimes implicated in human infections.

Virulent *V. cholerae* organisms:

- Live in salt water attached to algae, copepods or shells of crustaceans; if conditions become unfavourable, they become dormant and unculturable. Drinking contaminated water or vegetables washed with contaminated water can lead to epidemics in humans. Cycles of transmission are perpetuated when bacteria are shed into water sources by fecal contamination.
- Possess O1 or O-139 somatic antigens, and O1 isolates are serotyped as Inaba, Ogawa or Hokujima and as two biotypes, classical and El Tor.
- Cholera causes a voluminous watery diarrhea that is life-threatening because the patient becomes markedly dehydrated. After ingestion, the bacteria gain access to the epithelium of the small intestine, where they become attached and then secrete cholera toxin. Secretion of the toxin results in marked efflux of water and ions into the lumen of the intestine, which manifests as diarrhea and dehydration.
- For diagnosis of cholera, clinical signs in areas of endemicity or during epidemics are highly suggestive. Vibrios grow on culture media designed for enteric organisms, with added sodium chloride where appropriate. Feces samples are plated on thiosulphate-dtrate-bile softs-sucrose (TCBS) agar to obtain yellow colonies or BSA to get translucent colonies; Gram stain of these colonies reveals curved Gram-negative rods. In epidemics, feces can be examined for darting motility, which will be reduced by adding specific antiserum.
- Cholera can be prevented by proper treatment of drinking water. Cholera is treated with rehydration therapy (ORS) and, in severe cases, with tetracycline or doxycycline to shorten the course of the disease. Antibody to cholera toxin greatly reduces the severity of the disease.
- *Vibrio parahaemolyticus* is halophilic (has an exceptionally high requirement for sodium chloride) and is found in prawns and other seafood. It also produces an enterotoxin but its effect is much milder than that of cholera. Other vibrios (*Vibrio vulnificus*) occasionally cause human disease, including traumatic wound infections and, rarely, eye infections.

**Notes****TERMINAL QUESTIONS**

1. What is the Morphology of *Vibrio Cholerae* ?
2. What are the holding or transport media for *Vibrio Cholerae* ?

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Notes

3. Describe the serological classification of *Vibrio Cholerae* ?
4. What are the differences between Classical and EL TOR Vibrios ?
5. What is the mechanism of action of Cholera Toxin ?
6. What are the biochemical reactions for demonstration *Vibrio Cholerae* ?



ANSWERS TO INTEXT QUESTIONS

27.1

1. V. Cholerae
2. Darting Motility
3. Thiosulphate Citrate Bile Salt Medium(TCBS) and Bile salt agar (BSA).
4. H Antigen
5. They grow only on high salt containing medias

27.2

1. Incubation period is 24 hrs. to 5 days
2. Heat labile, toxin of E.Coli
3. They activates adenyl cyclase
4. Stool Sample and Rectal swab
5. By double enrichment
6. Thiosulphate Citrate Bile Salt Medium (TCBS) and Bile salt agar (BSA).