Unit- 2

Study of human diseases caused by enteric bacteria and spirochete

i) Salmonella typhi

 # Introduction: -

The genus salmonella consists of bacilli that parasitise the intestines of a large number of vertebrate species and infect human beings, leading to enteric fever, gastroenteritis, septicemia, and the carrier state.

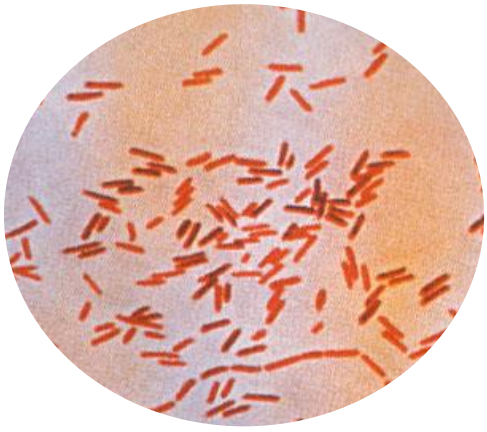
The most important member of the genus *Salmonella typhi*, the causative agent of typhoid fevers. The typhoid bacillus was first observed by Eberth (1880) in fatal cases of typhoid fever and was isolated by Gaffky (1884). Then Salmon and Smith (1885) studied typhoid-causing bacillus and named *Salmonella typhi.*

Salmonellae currently comprise above 2000 serotypes or species, all of them potentially pathogenic. For practical purposes, they may be divided into 2 groups:

1. **The enteric fever group: -** consisting of the typhoid and paratyphoid bacilli that are exclusively or primarily human parasites.
2. **The food poisoning group: -** produce gastroenteritis, septicemia or localised infections.

 # Morphology: -

-> *Salmonella typhi, salmonella paratyphi A, Salmonella paratyphi B* are Gram-negative rods, about 1-3µm x 0.5µm in size.
-> They are motile with peritrichate flagella.
-> They do not form capsules or spores but may possess fimbriae.



 # Cultural characteristics: -

- ➔ These are aerobic and facultative anaerobic
- ➔ Grow readily on simple media over a range of pH 6-8 and temperature 15-41 ^{0}C (optimum 37^{0}C).
- ➔ Colonies are large, 2-3 mm in diameter, circular, low convex and smooth. They are more translucent than coliform colonies.
- ➔ On MacConkey and Deoxycholate citrate media, colonies are colourless due to the absence of lactose fermentation.
- ➔ On Wilson and Blair Bismuth Sulphite medium, jet black colonies with a metallic sheen are formed due to production of H_2S. *S. paratyphi A* and others that do not form H_2S, produce green colonies.
- ➔ Selenite F and tetrathionate broth are commonly employed as enrichment media.

 # Biochemical reactions: -

Glucose	⊕
Mannitol	⊕
Maltose	⊕
Lactose	Negative
Sucrose	Negative
Salicin	Negative
Urease	Negative
Indole	Negative
MR	Positive
VP	Negative
Citrate	Positive
H_2S	Positive, except *S. paratyphi A.*

Following table shows differentiation between typhoid and paratyphoid bacilli on the basis of biochemical characteristics.

Sr. No.	Species	Glucose	Xylose	d-Tartrate
1	*S. typhi*	A	V	A
2	*S. paratyphi A*	AG	--	--
3	*S. paratyphi B*	AG	AG	--
4	*S. paratyphi C*	AG	AG	AG

AG ---- Acid & Gas
A ----- Acid
V ---- Variable

 # Resistance: -

- ➔ These are killed at 55^0C in one hour or at 60 ^{0}C in 15 minutes.
- ➔ Boiling or chlorination of water and pasteurisation of milk destroy the bacilli.
- ➔ In polluted water and soil, they survive for weeks and in ice for months.
- ➔ Cultures may be viable for years if prevented from drying.
- ➔ They are killed within 5 minutes by HgCl (1:500) or 5% phenol.

 # Antigenic structure: -

Salmonellae possess the following antigens based on which they are classified and identified.

1. Flagellar antigen H
2. Somatic antigen O
3. Surface antigen Vi

1. Flagellar antigen H: -

- ➔ This antigen present on the flagella is a heat labile protein destroyed by boiling or by treatment with alcohol but not by formaldehyde.
- ➔ When mixed with antisera, H suspensions agglutinate rapidly, producing large, loose, fluffy clumps.
- ➔ It is strongly immunogenic and induces antibody formation rapidly.

2. Somatic antigen O: -

- ➔ It is Phospholipid – protein – polysaccharide complex present in the cell wall.
- ➔ Unaffected by boiling, alcohol or weak acids.
- ➔ Form compact, chalky granular clumps when mixed with antisera.
- ➔ It is less immunogenic than H antigen.
- ➔ Fewer antibodies are produced against it.
- ➔ Salmonellae are classified into number of groups based on this antigen.

3. Vi antigen: -

- ➔ It is related to virulence hence name Vi for virulence.
- ➔ It is present on surface & may mask or block 'O' antigen.
- ➔ Heat labile, destroyed by 1N HCl and 0.5 N NaOH
- ➔ Not affected by alcohol or 0.2 % formol.
- ➔ The Vi antigen tends to be lost on serial subculture.
- ➔ The Vi polysaccharide acts as virulence factor by inhibiting phagocytosis, resisting complement activation.
- ➔ It is poorly immunogenic. The antibody disappears early. Its persistence indicates the development of carrier state.

 # Pathogenicity: -

Salmonellae are strict parasites of human and animals. *S. typhi, S. paratyphi A and S. paratyphi B* cause disease in man. Other species cause disease in animals.

Salmonellae cause the following clinical symptoms in man—

1. Enteric fever or Typhoid fever
2. Septicemia
3. Gastroenteritis or food poisoning

1. Enteric fever or Typhoid fever

- ➔ The term enteric fever includes- typhoid fever caused by *S. typhi, S. paratyphi A, S. paratyphi B and S. paratyphi C.*
- ➔ The infection is acquired by ingestion of these bacilli.
- ➔ The ID_{50} was found to be about 10^7 bacilli. Some times 10 bacilli are sufficient to produce disease.
- ➔ On reaching the gut, the bacilli attach to the epithelial cells of the intestinal villi and penetrate to the lamina propria & submucosa of intestine.
- ➔ They enter the mesentric lymph nodes & multiply.
- ➔ They enter the blood through thoracic duct. This is called bacteraemia.
- ➔ Then they multiply in the liver, gall bladder, spleen, bone marrow, lymph nodes, lungs and kidneys.
- ➔ As bile is a good culture medium for salmonellae, they multiply abundantly in the gall bladder and are continuously discharged in intestine. In intestine they infect Peyer's patches and lymphoid follicles of the ileum. Due to this inflammation occurs in the intestine and cells are damaged hence characteristic **'typhoid ulcers"** are formed (cracks or wounds in the intestine).

- ➔ Due to ulceration, intestinal perforation and haemorrhage occurs. (Perforation --> Hole in the walls of intestine, Haemorrhage --> Internal bleeding). After curing of disease in 3 to 4 weeks, ulcers are healed.
- ➔ Incubation period is usually 14 days but may range from 5 to 20 days.
- ➔ The severity of disease depends up on the number of bacilli, virulence of bacilli and immune response of the host body.

 # Symptoms: -

- ➔ Headache
- ➔ Malaise (Not feeling good well)
- ➔ Anorexia (loss of appetite for food)
- ➔ Coated tongue (Deposits on tongue)
- ➔ Abdominal discomfort
- ➔ Constipation or diarrhoea
- ➔ High temperature / fever 9Step ladder pyrexia)
- ➔ Bradycardia (slow or high pulse rate)
- ➔ Toxaemia (generalised poisoning of body due to toxin)
- ➔ Soft, palpable spleen
- ➔ Hepatomegaly (Enlargement of liver)
- ➔ Rose spots (red spots) on skin during 2^{nd} and 3^{rd} week

 # Important complications: -

- ➔ Intestinal perforations (holes in the walls of intestine)
- ➔ Haemorrhage (escape of blood from vessel)
- ➔ Circulatory failure
- ➔ Bronchitis or bronchopneumonia
- ➔ Psychoses (loss of control on thinking or mind)
- ➔ Deafness (less hearing)
- ➔ Meningitis (Inflammation of brain)
- ➔ Cholecystitis (Inflammation of gall bladder)
- ➔ Arthritis (Inflammation of joints)
- ➔ Abscesses (Localised collection of pus)
- ➔ Periosteitis (Inflammation of membrane which cover long bone)
- ➔ Nephritis (Inflammation of kidney)
- ➔ Haemolytic anaemia (Deficiency of blood due to destruction of RBCs)
- ➔ Venous thrombosis (Intravascular formation of blood clot)
- ➔ Peripheral neuritis (Inflammation of nerves)
- ➔ Rare Osteomyelitis (Inflammation of bone marrow)

  S. paratyphi A and B cause paratyphoid fever which is similar to typhoid but it is generally not so severe like typhoid.

 # Epidemiology: -

- ➔ Typhoid fever is nearly eliminated from the developed countries mainly as a result of improvements in water supply and good sanitation but it continues to be endemic in the poor nations of the world.
- ➔ The distribution of paratyphoid bacilli shows marked geographical differences. *S. paratyphi A* prevalent in India and other Asian countries, Eastern Europe and South America. *S. paratyphi B* in western Europe, Britain and North America; and *S. paratyphi C* in Eastern Europe and Guyana.
- ➔ Enteric fever is endemic in all parts of India. Though there is no reliable information about its incidence in the country, typhoid fever has been estimated to affect 150-300 per 100, 000 population.
- ➔ The proportion of typhoid to paratyphoid A is about 10:1. Paratyphoid B is rare and C very rare.
- ➔ The disease occurs at all ages but is probably most common in the 5-20 year age group.
- ➔ The source of infection is a patient, or far more frequently, a carrier. Patients who continue to shed typhoid bacilli in feces for 3 weeks to 3 months after clinical cure are called **'convalescent carriers'.** Those who shed the bacilli for more than 3 months but less than a year are called **'temporary carriers'** and those who shed the bacilli for over a year are called **'chronic carriers'.** About 2-4 % patients become chronic carriers.
- ➔ The development of the carrier state is more common in women and in the older age groups (over 40 years). The shedding of bacilli is usually intermittent. The bacilli persist in the gall bladder or kidney and are eliminated in feces or urine.
- ➔ **Mary Mallon (Typhoid Mary): -** Food handlers or cooks who become carriers are particularly dangerous. The best known of such typhoid carriers was Mary Mallon, a New York cook lady who, over a period of 15 years, caused at least 7 outbreaks affecting over 200 persons.
- ➔ Carriers occur with paratyphoid bacilli also. While *S. paratyphi A* occurs only in human beings, *S. paratyphi B* can infect animals such as dogs or cows, which may act as sources of human disease.
- ➔ Typhoid fever occurs in 2 epidemiological types. The forst is **endemic or residual typhoid** that occurs throughout the year. The second is **epidemic.** Typhoid epidemics are water, milk or food borne.
- ➔ The rate of clinical typhoid is about 25 times higher in the HIV infected than in others.

 # Laboratory diagnosis: -

It is done by following methods—

1. Isolation of bacilli from blood, faeces, urine and other materials.
2. Demonstration of antibodies by Widal test
3. Demonstration of circulating antigen
4. Other laboratory tests
5. Bacteriophage typing

1. Isolation of bacilli from blood, faeces, urine and other materials: -

Blood culture: - Bacteremia occurs early in the disease and blood cultures are positive in approximately 90% of cases in the first week of fever, 75% in the second week, 60% in the third week and 25% thereafter till the subsidence of pyrexia (fever).

About 5-10 ml of blood is collected by venepuncture and inoculated into a culture bottle containing 50-100 ml of 0.5% bile broth. To prevent bactericidal action of blood, Sodium polyanethol sulphonate is added. After incubation at 37^0C for 24 hours, the bile broth is subcultured on MacConkey's agar. Pale nonlactose fermenting colonies that may appear on this medium are picked out for biochemical tests and motility. Isolated bacilli are also tested for agglutination with the O antiserum.

For the identification, the help of the National Salmonella Reference Centre situated at Central Research Institute, Kasauli, India is taken.

Castaneda's method: - To eliminate the risk of introducing contamination during repeated subcultures, and also for economy and safety, Castaneda's method of culture may be adopted. In this method, a double medium is used. The bottle of bile broth has an agar slant on one side. After inoculation of blood, the bottle is incubated in the upright position. For subculture, the bottle is merely tilted so that the broth runs over the surface of the agar. It is reincubated in upright position. If salmonellae are present, colonies will appear on the slant.

Faeces culture: - Salmonellae are shed in feces throughout the course of the disease and even in convalescence, with varying frequency. Hence faecal cultures are almost as valuable as blood cultures in diagnosis. A positive faecal culture, however, may occur in carrier as well as in patients. Faecal samples are plated directly on MacConkey's, DCA and Wilson-Blair media. Further procedure is same like used for blood cultures.

Urine culture: - Salmonellae are shed in the urine irregularly and infrequently. Hence urine culture is less useful. Cultures are positive only in the second and third week. Procedure for isolation is same like blood and faeces culturing.

Other material for culture: - Culture of bone marrow, bile, rose spots, pus from suppurative lesions, CSF and sputum, gall bladder, liver, spleen and mesenteric lymph nodes can be used.

2. Demonstration of antibodies by Widal test: -

- ➔ This is a test for measurement of H and O antibodies for typhoid and paratyphoid bacilli in the patient's sera.
- ➔ Two types of tubes are generally used for the test – a narrow tube with a conical bottom (Dreyer's agglutination tube) for H agglutination and a short round-bottomed tube (Felix tube) for the O agglutination.
- ➔ Equal volumes (O.4 ml) of serial dilutions of the serum (from 1/10 to 1/640) and the H and O antigens are mixed in Dreyer's and Felix agglutination tubes, respectively and incubated in a water bath at 37^0C overnight. Some workers recommend incubation at 50-55 ^{0}C for 2 hours, followed by overnight incubation at room temperature.
- ➔ Control tubes containing the antigen and normal saline are set to check for agglutination.
- ➔ The agglutination titres of the serum are read. H agglutination leads to the formation of loose, cotton woolly clumps, while O agglutination is seen as a disc-like pattern at the bottom of the tube.
- ➔ The antigens used in the test are the H and O antigens of *S. typhi* and the H antigens of *S. paratyphi A and B.*
- ➔ The paratyphoid O antigens are not employed as they cross react with the typhoid O antigen due to their sharing of factor 12.
- ➔ The results of the Widal test should be interpreted taking into account the following—
    - The agglutination titre will depend on the stage of the disease. Antibodies usually appear by the end of the first week, so that blood taken earlier may give a negative result. The titre increases steadily till the third or the fourth week, after which it declines gradually.
    - Demonstration of a rise in titre of antibodies, by testing two or more serum samples, is more meaningful than a single test.
    - It is generally stated that titres of 1/100 or more for O antibodies and 1/200 or more for H antibodies are significant.
    - Serum from an individual immunised with TAB vaccine will generally have antibodies to typhoid bacilli.
    - Cases treated early with chloramphenicol or other antibiotic show a poor agglutinin response.
- ➔ Other serological methods of diagnosis include indirect haemagglutination, CIE and ELISA.

3. Demonstration of circulating antigen: -

- ➔ Typhoid bacillus antigens are consistently present in the blood in the early phase of the disease, and also in the urine of the patients.
- ➔ The antigen can be demonstrated by sensitised Staphylococcal coagglutination test.
- ➔ *Staphylococcus aureus (Cowan I strain), which* contains protein A, is stabilised with formaldehyde and coated with *S. typhi* antibody.
- ➔ When a 1% suspension of such sensitised staphylococcal cells is mixed on a slide with serum from patients in the first week of typhoid fever, the typhoid antigen present in the serum combines with the antibody attached to staphylococcal cells producing visible agglutination within 2 minutes.
- ➔ The test is rapid, sensitive and specific but is not positive after the first week of the disease.

4. Other laboratory tests: -

- ➔ A WBC count is useful. Leucopenia with a relative lymphocytosis is seen. Eosinophils are said to be absent but in the tropics, with a high incidence of helminthic infestation, eosinophils are usually present.
- ➔ **Diazo test: -** Equal volumes of the patient's urine and the diazo reagent are mixed in a test tube and a few drops of 30% NH_4OH added. On shaking a mixture, froth develops, which is red or pink, if the test is positive. The diazo reaction becomes positive usually between the 5^{th} and 14^{th} days of fever and becomes negative when the fever subsides. This test may be helpful where bacteriological or serological facilities are not available.

5. Bacteriophage typing: -

- ➔ Intraspecific classification of *S. typhi* for epidemiological purposes was made possible by bacteriophage typing. At present 97 Vi II phage types of *S. typhi* are recognised.

 # Prophylaxis: -

- ➔ Typhoid fever can be effectively controlled by general measures, such as improvements in sanitation and provision of protected water supply.
- ➔ Specific prophylaxis with heat killed typhoid bacillus vaccine was developed and successfully field-tested by **Almroth Wright** during the Boer war in South Africa.
- ➔ **TAB vaccination: -** The TAB vaccine which came into general use later contains *S. typhi*, 1000 million, *S. paratyphi A*, 750 million and *S. paratyphi*

B 750 million bacteria per ml killed by heating at 50-60 ^{0}C and preserved in 0.5% phenol.

- ➔ The vaccine is given in two doses of 0.5 ml subcutaneously at an interval of 4-6 weeks.
- ➔ In endemic areas vaccination is recommended for all. Booster doses are repeated in such areas.
- ➔ **The live oral vaccine (Typhoral): -** It is a stable mutant of *S. typhi* strain Ty2 la, lacking the enzyme UDP-galactose-4-epimerase (Gal E mutant). On ingestion, it initiates infection but 'self destructs' after 4 or 5 cell divisions, and therefore cannot induce any illness. The vaccine is an enteric-coated capsule containing 10^9 viable lyophilised mutant bacilli. The course consists of one capsule orally, taken an hour before food, with a glass of water or milk, on days 1, 3, and 5. No antibiotic should be taken during this period.
- ➔ **The injectable vaccine (Typhim- Vi): -** It contains purified Vi polysaccharide antigen (25 µg per dose) from *S. typhi strain* Ty2. It is given as a single subcutaneous or intramuscular injection, which causes only minimal local reaction.
- ➔ Both these vaccines are recommended only in those over 5 years of age, the same dose being used for children and adults. In both cases protection is stated to commence 2-3 weeks after administration and lasts for at least 3 years, after which a booster may be given.

 # Treatment: -

- ➔ Specific antibacterial therapy for enteric fever became available only in 1948 with the introduction of **Chloramphenicol** antibiotic, which continued as the sheet anchor against the disease till the 1970s when resistance became common.
- ➔ Though *S. typhi* is susceptible in vitro to many antibiotics such as Streptomycin, Tetracycline etc, it is ineffective in vivo.
- ➔ Ampicillin, Amoxycillin, Furazolidone and Cotrimoxazole were other drugs that had been found useful in the treatment of typhoid fever.
- ➔ At present, the drugs useful in the treatment of multi drug resistant typhoid cases are the later fluroquinolones such as Ciprofloxacin, Pefloxacin, Ofloxacin, Gaietyfloxacin and the third generation cephalosporins such as Ceftazidime, Ceftrioxone, and Cefotaxime.

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ii) *Vibrio cholerae*

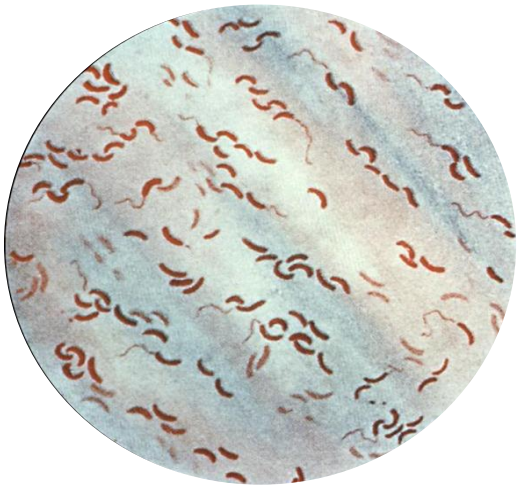
Vibrio

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 nocapsulated. 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 had been observed earlier by Pacini (1854) and others.

Vibrio cholerae

- **Morphology**

The cholera vibrio is a short, curved, cylindrical rod, about 1.5 µm x 0.2-0.4 µm 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 forms may be seen due to two or more cells lying end to end. Pleomorphism is frequent in old cultures. In stained films of mucous 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. The vibrios stain readily with aniline dyes and are gram negative.



- **Cultural characteristics**

The cholera vibrio is strongly aerobic, growth being scanty and slow anaerobically. It grows within a temperature range of 16-40^0 (optimum 37^0 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 concentrations (6% and above) are inhibitory.

It grows well on ordinary media on nutrient agar. After overnight growth, colonies are moist, translucent round disks, about 1-2 mm in diameter, with a bluish tinge in transmitted light. The growth has a distinctive odour. 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 becomes clear due to hemodigestion. In gelatin stab culture, infundibuli form (funnel shaped) or napiform (turnip shaped) liquefaction occurs in three days at 22^{0}C. In peptone water, growth occurs in about six hours as a fine surface pellicle, which on shaking breaks up into membranous pieces. Turbidity and a powdery deposit develop on continued incubation.

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

Holding or transport Media:

1) Venkatraman-Ramakrishnan (VR) medium: A simple modified form of this medium is prepared by dissolving 20 g crude sea salt and 5 g peptone in one liter of distilled water and adjusting the pH to 8.6-8.8. It is dispensed in screw capped bottles in 10-15 ml amounts. About 1-3 ml stool is to be added to each bottle. In this medium vibrios do not multiply but remain viable for several weeks.

2) Cary-Blair medium: This is a buffered solution of sodium chloride, sodium thioglycollate, disodium phosphate and calcium chloride at pH 8.4.

3) Autoclaved sea water also serves as a holding medium.

Enrichment Media:

1) Alkaline peptone water at pH 8.6.

2) Monsur's taurocholate tellurite peptone water at pH9.2.

Both these are good transport as well as enrichment media.

Plating media:

1) Alkaline bile salt agar (BSA) pH 8.2: - This simple medium has stood the test to time and is still widely used. The colonies are similar to those on nutrient agar.

2) Monsur's gelatin taurocholate trypticase tellurite agar (GTTA) medium: Cholera vibrios produce small, translucent colonies with a grayish black center and a turbid halo. The colonies become 3-4 mm in size in 48 hours.

3) TCBS medium: This medium containing thiosulfate, cirtrate, bile salts and sucrose, is available commercially and is very widely used at present cholera vibrios produce large yellow convex colonies which may becomes green on continued incubation.

String test: - Vibrio colonies may be identified by the string test. A loopful of the growth is mixed with a drop of 0.5% sodium deoxycholate in saline on a slide. If the test is positive, the suspension loses its turbidity, becomes mucoid and forms a 'string' when the loop is drawn slowly away from the suspension.

- **Biochemical reactions**

Carbohydrate metabolism is fermentative, producing acid, but no gas. Cholera vibrios ferment

Glucose	+
Mannitol	+
Maltose	+
Mmannose	+
Sucrose	+
Inositol	_

Arabinose	_
Lactose	_ though lactose may be split very slowly
Indole	+
Nitrate reduction	+
Catalase	_
Oxidase	_
Lysine and Ornithine decarboxylation	+
Arginine decarboxylation	_
Gelatin liquification	+

Cholera Red Reaction: - Indole production and Nitrate reduction 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 is developed due to the formation of nitroso-indole.

- **Resistance**

Cholera vibrios are susceptible to heat, drying and acids, but resist high alkalinity. They are destroyed at 55^0 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, termperature, sanlinity, presence of organic pollution and other factors. In general, the El 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. In grossly contaminated water, such as the Ganges water of India, the vibrios do not survive for any length of time, due to the apparently large amounts of vibriophages present. They survive in clean tap water for thirty days. In untreated night soil, they may survive for several days. Vibrios are susceptible to the common disinfectants.

On fruits 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 harbor 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 a chlorhydric gastric juice.

- **Classification**

In the past, many oxidase positive, motile, curved rods were rather loosely grouped as vibrios. Precise criteria have been laid down for differentiating vibrios from related genera.

Heiberg (1934) classified vibrios into six groups based on the fermentation of mannaose, sucrose and arabinose. Two more groups were added later. Cholera vibrios belong to Group I.

Gardner and Venkatraman: - A serological classification was introduced by Gardner and Venkatraman (1935) Cholera vibrios and biochemically similar vibrios, possessing a common flagellar (H) antigen were classified as Group A vibrios and the rest as Group B vibrios comprising a heterogeneous collection. Based on the major somatic (o) antigen. Group A vibrios were classified into subgroups (now called O serogroups or servoars), 139 of which are currently known. Pathogenic cholera vibrios (which are referred to as 'agglutinable vibrios'). Other vibrio isolates which were not agglutinated by the O-1 antiserum came to be called nonagglutinable or NAG vibrios. They were considered nonpathogenic and hence also called noncholera vibrios. (NCV.) .

While all isolates from epidemic cholera belonged to group O-1, not all members of the group were capable of causing clinical cholera. The first such members whish acquired prominence were the vibrios isolated by Gottschlich (1905) from six Hajpilgrims who died at the Tor quarantine station on the Sinai Peninsula. They had died not from cholera but from dysentery or gangrene of the colon. These came to be called the El Tor vibrios, They were identical to cholera vibrios in all laboratory tests except that they were hemolytic to sheep erythrocytes and gave a positive Voges-Proskauer reaction.

Based on minor surface antigenic characteristics both classical and E1 Tor biotypes of cholera vibrios were classified into three serotypes, Ogawa, Inaba and Hikojima. The ogawa and Inaba strains are agglutinated by their own respective specific sera only, while the Hikojima strains are agglutinated by both ogawa and Inaba antisera. There serotypes sertoping is only of epidemiological significane.

- **Cholera Symptoms**

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 effortless vomiting may lead to hypovolemic shock and death in less than 24 hours. In treated cases, the disease may pass a total volume of liquid stool equal to twice his body weight. 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') It has a characteristic inoffensive sweetish odour. In composition it is a bicarbonate-rich isotonic electrolyte solution, with little protein. Its outpouring leads to diminution of extracellular fluid volume, hemoconcentration, hypokalemia, base-deficit acidosis and shock.

The common complications are muscular cramps, renal failure, pulmonary edema, cardiac arrhythmias and paralytic ileus. 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 E1 Tor vibrios than with the classical 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**

Natural infection with cholera occurs only in humans and not in animals. In human infection, the virbios enter orally through contaminated water or food. Vibrios are highly susceptible to acids. And gastric acidity provides an effective barrier against small does of cholera vibrios.

In the small intestine, vibrios are enabled to cross the protective layer of mucus and reach the eptithelial cells by chemotaxis, motility, mucinase and other protelytic enzymes. A hemagglutnin protease (formerly known as cholera lectin) cleaves mucus and fibronectin. It also helps in releasing vibrios bound to bowel mucosa, facilitating their spread to other parts of the intestine and also their fecal shedding Adhesion to the epithelial surface and colonization may be

facilitated by special fimbriae such as the toxin co regulated pilus (TCP) Throughout the course of infection, the vibrios remain attached to the epithelium but do not damage or invade the cells. The changes induced are biochemical rather than histological.

Vibrios multiplying on the intestinal epithelium produce a toxin (choleragen, cholera enterotoxin, cholera toxin, CT, or CTX) which is very similar to the heat labile toxin (LT) of E. coli in structural, chemical, biological and antigenic properties, though CT is far more potent that LT in biological activity.

The toxin molecule, of approximately 84,000 MW consists of one A and 5 B subunits. The B (binding) units attach to the GM1 ganglioside receptors on the surface of jejunal epithelial cells. The A (active) submit. On being transported into the enterocyte dissociates into two fragments A1 and A2. The A2 fragment only links the biologically active A1 to the B subunit. The A1 fragment causes prolonged activation of cellular adenylate cyclate and accumulation of cAMP, leading to outpouring into the small intestinal lumen, of large quantities of water and electrolytes and the consequent water diarrhoea. The fluid secreted is isotonic with plasma but contains much more of potassium and bicarbonate. The toxin also inhibits intestinal absorption of sodium and chloride. All clinical manifestations and complications in cholera result from the massive water and electrolyte depletion thus caused.

- **Epidemiology**

Cholera can occur in many forms-sporadic, endemic, epidemic or pandemic. India, more specifically the large deltaic area of the Ganges and Brahmaputra in Bengal, is its homeland, where it has been known from very ancient times. Till early in the nineteenth century, Cholera was virtually confined to India, periodically causing large epidemics in different parts of the country.

From 1817 to 1923 cholera vibrios had spread from Bengal, in six separate pandemic waves, involving most parts of the world. It was largely due to the threat of pandemic cholera that international health organizations came into being. After the end of the 6 th pandemic in 1923, till 1961 the disease remained con fined to its endemic areas, except for an isolated epidemic in Egypt in 1947.

The seventh pandemic originated from sulawesi (Celebes), Indonesia, in 1961 when the E1 Tor vibrios which had been smoldering there for many decades suddenly became more virulent. After spreading to Hongkong and the Philipines, it spread steadily westwards, invading India in 1964. By 1966, it had spread throughout the Indian, subcontinent and west asia. In the 1970s the pandemic extended to Africa and parts of Southern Europe.

During the course of the Pandemic, the vibrios had invaded affluent countries also. However, they remained localized and were soon controlled, in contrast to the outbreaks in the poor nations, which developed into prolonged and extensive epidemics.

By 1994 most parts of central and South America had been involved and rendered endemic.

Cholera is an exclusively human disease. Infection originates from the patient or the carrier. Carriers may be incubatory, convalescent, healthy or chronic. Incubatory carriers shed vibrios only during the brief incubation period of 1-5 days. Convalescents may excrete them for 2-3 weeks. The healthy or contact carrier who has had subclinical infection usually sheds the vibrios for less than 10 days. The chronic carrier continues to be active for months or years the longest duration recorded being 10 years.

Infection is acquired through faecally contaminated water or food, Direct person-to-person spread by contact may not be common but hand contamination of stored drinking water has been shown to be an important method of domestic spread of infection.

A significant difference is susceptibility to cholera has been reported in relation to blood groups group O persons being the most and group AB the least susceptible. The reason for this is not known.

- **Laboratory diagnosis**

Isolation of cholera vibrios from stools is a simple matter as they are present in very large numbers 10^6-10^9 vibrios per ml. The specimen is best collected by introducing into the rectum a lubricated catheter and letting the liquid stool flow directly into a screw capped container. Vomitus is not useful.

As cholera vibrios may die in a few hours at tropical temperatures, it is necessary to preserve the specimen at 4^{o} C or in some appropriate holding medium. Stool samples may be preserved in VR fluid or Cary Blair medium for long periods. Whenever possible, specimens should be plated and the inoculated plates sent to the laboratory. The plating media used vary in different laboratories but the media employed usually are bile salt agar, MacConkey agar for nonselective and TCBS agar for selective plates. Colonies suggestive of vibrios should be picked with a straight wire and tested by slide agglutination with cholera O subgroup I serum (cholera 'non-differential' serum). If positive, the isolate is tested for chick red cell agglutination. This is employed for presumptive differentiation between E1 Tor and classical cholera vibrios.

Diagnosis by direct microscopic examination of cholera stool is not recommended as the results are not reliable. For rapid diagnosis, the characteristic motility of the vibrio and its inhibition by antiserum can be demonstrated under the dark field or phase contrast microscope, using cholera stool from acute cases, or more reliably after enrichment for six hours.

Serological examination is of little use in the diagnosis of cases though it may be helpful in assessing the prevalence of cholera in an area.

- **Prophylaxis.**

The prevention of cholera requires essentially general measures such as provision of protected water supply and improvement of environmental sanitation. As these are not easily attainable, vaccination continues to be the most widely used method of prevention endemic areas.

Cholera vaccines were introduced by Ferran with in a year of the discovery of the vibrio. The original vaccines were live suspensions of vibrios. As they gave rise to adverse reactions, they were replaced by killed suspensions containing 8000 millions V. cholerae per ml. Composed of equal numbers of ogawa and Inaba serotypes, given by subcutaneous or intramuscular injection. Many laboratories employ classical cholera and E1 Tor vibrios in equal numbers in the vaccine. Strain O139 vaccine has also been prepared. The concentration of the vaccine has been increased to 12,000 million per ml, in order to improve the antigenic stimulus.

The duration of protection is only 3-6 months; the rate of protection in endemic areas increases with age; a single does of vaccine is infective in children below five year of age while two doses at 1-4 week intervals are protective; a single does confers good protection in adults due to its acting as a booster on top of prior natural immunization. Injectable vaccines do not provided any local immunity in the intestinal mucosa. Hence attention has been directed to oral vaccines.

Two types of oral vaccines have been tried recently-killed oral whole cell vaccines with a without the inclusion of the B subunit of CT, and live oral vaccines with classical, E1 Tor and O-139 strains, with their toxin genes deleted. While the results have been promising, problems remain to be solved before they are cleared for general use.

An ideal cholera vaccine is yet to be found. Cholera vaccination was a compulsory requirement for international travel, but now very few countries insist on this.

- **Treatment**

The treatment of cholera consists essentially of the prompt and adequate replacement of lost fluid and electrolytes. Oral administration of fluid containing glucose and electrolytes, either alone or supplemented by intravenous fluid is a highly successful and freely available method of treating cholera. Cereal based preparations are equally effective and usually more acceptable.

Antibacterial therapy is of secondary importance. Oral tetracycline was recommended for reducing the period of vibrio excretion and the need for parenteral fluids. Initially cholera vibrios were uniformly susceptible to all antibiotics active against Gram negative bacilli, but since 1979, multiple drug resistant strains have become increasingly common.

Now a day Ciprofloxacin, Ofloxacin, Sparfloxacin, Chloramphenicol, antibiotics are used.

iii) *Treponema pallidum* (Syphilis)

Treponema pallidum is a Gram-negative bacterium which is spiral in shape. It is an obligate internal parasite which causes syphilis, a chronic human disease. **Syphilis** is a sexually transmitted infection caused by the spirochete bacterium *Treponema pallidum* subspecies *pallidum*. Syphilis is a sexually transmitted disease but transmission can also occur between mother and child in utero; this is called congenital syphilis. Syphilis was first discovered in Europe near the end of the fifteenth century. The virulent strain of *T. pallidum* was first isolated 1912.

- **Morphology**

T. pallidum is a Gram-negative bacteria consisting of an inner membrane, a thin peptidoglycan cell wall, and an outer membrane. It is very small in size with a length that ranges from 6 to 20 um and a diameter range of 18-20 um. *T. pallidum* is a member of the spirochete family which are characterized by their distinct helical shape. Probably the most interesting property of T. pallidum's structure is the endoflagella found in the periplasmic space between its two membranes. These organelles give *T. pallidum* its distinctive corkscrew motility.

- **Cultural characteristics**

T. pallidum is also microaerophilic, meaning that it requires a very low concentration of oxygen. It is an obligate internal parasite, meaning that it requires a mammalian host for survival. In the absence of mammalian cells, *T. pallidum* will be killed by the absence of nutrients, exposure to oxygen and heat. Since *T. pallidum* cannot be grown in culture, animal models are needed to study syphilis. Although mice and monkeys can be used, rabbits are the animal model almost exclusively studied in the lab.

- **Pathogenecity**

T. pallidum is the causative agent of syphilis, a chronic infectious human disease transmitted between individuals via sexual intercourse or from mother to child in utero. *T. pallidum*'s virulence factor is still unknown. Syphilis can present in one of four different stages:

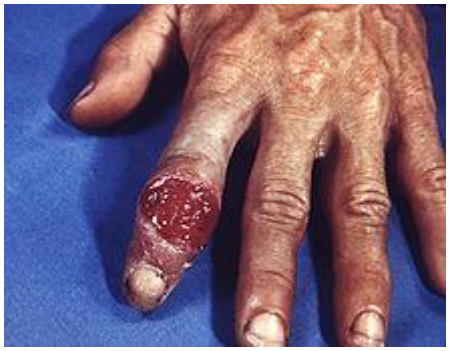
i) Primary

ii) Secondary,

iii) Latent

iv) Tertiary.

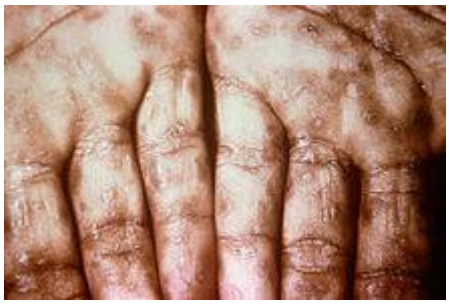
i) Primary



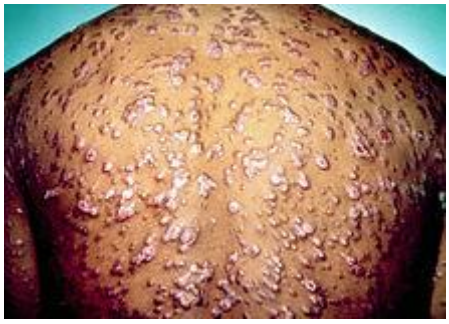
Primary chancre of syphilis on the hand

Primary syphilis is typically acquired by direct sexual contact with the infectious lesions of another person. Approximately three to 90 days after the initial exposure (average 21 days) a skin lesion, called a chancre, appears at the point of contact. Chancre is classically single, firm, painless, non-itchy skin ulceration with a clean base and sharp borders between 0.3 and 3.0 cm in size. The lesion, however, may take on almost any form. In the classic form, it evolves from a macule to a papule and finally to an erosion or ulcer. Occasionally, multiple lesions may be present with multiple lesions more common when coinfected with HIV. Lesions may be painful or tender and they may occur outside of the genitals. The most common location in women is the cervix, the penis in men occurring 7–10 days after chancre formation.

ii) Secondary



Typical presentation of secondary syphilis with a rash on the palms of the hands



Reddish papules and nodules over much of the body due to secondary syphilis

Secondary syphilis occurs approximately four to ten weeks after the primary infection. While secondary disease is known for the many different ways it can manifest, symptoms most commonly involve the skin, mucus membranes, and lymph nodes. There may be a symmetrical reddish-pink non-itchy rash on the trunk and extremities, including the palms and soles.

Other symptoms may include fever, sore throat, malaise, weight loss, hair loss, and headache. Rare manifestations include hepatitis, kidney disease, arthritis, periostitis, optic neuritis, uveitis, and interstitial keratitis.

iii) Latent

Latent syphilis is defined as having serologic proof of infection without symptoms of disease. Early latent syphilis may have a relapse of symptoms. Late latent syphilis is asymptomatic, and not as contagious as early latent syphilis.

iv) Tertiary

Tertiary syphilis may occur approximately three to 15 years after the initial infection, and may be divided into three different forms: gummatous

syphilis, late neurosyphilis, and cardiovascular syphilis. Without treatment, a third of infected people develop tertiary disease. People with tertiary syphilis are not infectious.

Gummatous syphilis or late benign syphilis usually occurs one to 46 years after the initial infection, with an average of 15 years. This stage is characterized by the formation of chronic gummas, which are soft, tumor-like balls of inflammation which may vary considerably in size. They typically affect the skin, bone, and liver, but can occur anywhere.

Neurosyphilis refers to an infection involving the central nervous system. It may occur early, being either asymptomatic or in the form of syphilitic meningitis, or late as meningovascular syphilis, which is associated with poor balance and lightning pains in the lower extremities. Late neurosyphilis typically occurs four to 25 years after the initial infection.

Cardiovascular syphilis usually occurs 10–30 years after the initial infection.

Congenital

Congenital syphilis may occur during pregnancy or during birth. Two-thirds of syphilitic infants are born without symptoms. Common symptoms that then develop over the first couple years of life include: hepatosplenomegaly, rash, fever, neurosyphylis, and pneumonitis. If untreated, late congenital syphilis may occur including: saddle nose deformation.

- **Epidemiology**

Syphilis is transmitted primarily by sexual contact or during pregnancy from a mother to her fetus; the spirochaete is able to pass through intact mucous membranes or compromised skin. It is thus transmissible by kissing, oral, vaginal, and anal sex. Approximately 30 to 60% of those exposed to primary or secondary syphilis will get the disease. Its infectivity is exemplified by the fact that an individual inoculated with only 57 organisms has a 50% chance of being infected. It can be transmitted via blood products. However, it is tested for in many countries and thus the risk is low. The risk of transmission from sharing needles appears limited. Syphilis cannot be contracted through toilet seats, daily activities, hot tubs, or sharing eating utensils or clothing.

- **Laboratory diagnosis**

Syphilis is difficult to diagnose clinically early in its presentation. Confirmation is either via blood tests or direct visual inspection using

microscopy. Blood tests are more commonly used, as they are easier to perform. Diagnostic tests are, however, unable to distinguish between the stages of the disease.

Blood tests

Blood tests are divided into nontreponemal and treponemal tests. Nontreponemal tests are used initially, and include

i) Venereal Disease Research Laboratory (VDRL) test

ii) Rapid Plasma Reagin (RPR) test.

i) VDRL test

The Venereal Disease Research Laboratory test or VDRL is a blood test for syphilis and was developed by the former Venereal Disease Research Laboratory, of the United States Public Health Service. The basis of the test is that an antibody produced by a patient with syphilis reacts with an extract of ox heart (diphosphatidyl glycerol). It therefore detects anti-cardiolipin antibodies (IgG, IgM or IgA), visualized through foaming of the test tube fluid, or "flocculation". VDRL test is performed by mixing the VDRL antigen with heat-inactivated patient's serum on a glass slide. The mixture is then rotated either mechanically or manually for four minutes and later examined for the presence or absence of clumping. A strongly positive VDRL gives the appearance of floccules due to the aggregation of antigen (rod-shaped particles), formation of small clumps is indicative of weak reaction. A negative result is indicated if the antigens remain unchanged; it means antigen appears as short, rod-shaped "particles" at 100 X magnification.

Requirements: -

1) VDRL antigen (commercially available)
2) 0.2 ml serological pipettes graduated in 0.01 ml (4)
3) Heat inactivated syphilatic (reactive) serum 4^{+} (positive control)
4) Heat inactivated non-reactive serum (negative control)
5) Flat clear glass slide with several ceramic or paraffin rings approximately 14 mm in diameter.
6) Buffered saline solution (commercially available)

Procedure: -

1) Number 5 rings on the glass slide
2) Pipette 0.05 ml of the 4^{+} serum 2 seramic rings 1 & 4 using and fresh pipette.
3) With another pipette, add 0.05 ml of non-reactive serum to seramic rings 2 & 5
4) Add 0.05 ml of buffered saline to rings 3, 4 & 5.
5) Add 1 drop (1/60 ml) of VDRL antigen into the rings 1, 2 and 3.
6) Immediately after addition of the antigen, rotate the slide on a flat surface for four (4) minutes on a rotating machine or rotate by hand making a circle 2 inch in diameter 120 times / minute for 4 minutes.
7) Examine the slide immediately after rotation under low power objective (10 X) of your microscope (*i.e.* 100 X magnification).

ii) RPR test

Rapid Plasma Reagin (RPR) refers to a type of test that looks for non-specific antibodies in the blood of the patient that may indicate that the organism (*Treponema pallidum*) that causes syphilis is present. The term "reagin" means that this test does not look for antibodies against the actual bacterium, but rather for antibodies against substances released by cells when they are damaged by *T. pallidum*. The RPR test is an effective screening test, as it is very good at detecting people without symptoms who are affected by syphilis.

This test is based on the relationship between the lipid extract of tissue i.e. cardiolipin-cholesterol-lecithin. These lipids act as antigen against a non-specific antibody (reagin) which develops in the blood serum of syphilis affected persons. RPR is useful as a rapid screening test for syphilis patients. In this test only small amount of serum or plasma is used. Prior inactivation of serum or dilution of antigen is not required.

RPR test is modified version of Wasserman's reaction in which the antigens coated with the particle are allowed to react with the sample and if the antibodies for syphilis are present, the flocculation will occur on the slide due to aggression of carbon particles will form. Aggregate in the center of slide will be formed to give appearance of button which indicates negative reaction.

Requirements:

Reagent 1 – R.P.R. Antigen

Reagent 2- Positive control

Reagent 3- Negative control

Accessories- Plastic droppers, mixing sticks, rubber teat, delivery dropper, plastic slides.

Procedure: Screening Test

1. Place one drop of serum/plasma (50 µl) on the slide with disposable serum dropper.
2. After gently mixing R.P.R. antigen suspension, place one drop (10-20 µl) by antigen dropper.
3. Mix well and spread out the liquid o entire area of the circle by using disposable mixing stick.
4. Rock the slide gently for 6 minutes and observe under good light source for appearance of carbon particle clumping.

- **Prophylaxis**

There is no vaccine effective for prevention. Abstinence from intimate physical contact with an infected person is effective at reducing the transmission of syphilis, as is the proper use of a latex condom. Condom use, however, does not completely eliminate the risk. Congenital syphilis in the newborn can be prevented by screening mothers during early pregnancy and treating those who are infected.

- **Treatment**

Early infections

The first-choice treatment for uncomplicated syphilis remains a single dose of intramuscular penicillin G or a single dose of oral azithromycin. Doxycycline and tetracycline are alternative choices; however, they cannot be used in pregnant women. Antibiotic resistance has developed to a number of agents, including macrolides, clindamycin, and rifampin. Ceftriaxone, a third-generation cephalosporin antibiotic, may be as effective as penicillin-based treatment.

Late infections

For neurosyphilis due to the poor penetration of penicillin G into the central nervous system, those affected are recommended to be given large doses of intravenous penicillin for a minimum of 10 days. If a person is allergic, ceftriaxone may be used or penicillin desensitization attempted. Other late presentations may be treated with once-weekly intramuscular penicillin G for three weeks. If allergic, as in the case of early disease, doxycycline or tetracycline may be used, but for a longer duration.

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