

Definition: an illness acquired

Food poisoning:

through consumption of food/drink
contaminated either with micro-organisms or their toxins.

• **1-6 h IP:** pathogens liberate preformed toxins in food

• **8-16 h IP:** pathogens produce toxin in intestine after consumption of food

• **> 16 h IP:** > 24h after consumption of contaminated food

Microbial Agents Causing Food Poisoning:

Organisms	Symptoms	Common food sources
1-6h incubation period (IP)		
• <i>Staphylococcus aureus</i>	vomiting, diarrhoea, abdominal cramps	Ham
• <i>Bacillus cereus</i>	vomiting, diarrhoea, abdominal cramps	Fried rice
8-16h IP		
• <i>Clostridium perfringens</i>	diarrhoea, abdominal cramps	Poultry, beef
• <i>Bacillus cereus</i>	diarrhoea, abdominal cramps	Meat, dried beans
> 16h IP		
• <i>Vibrio cholerae</i>	Watery diarrhoea	Shellfish
• Enterotoxigenic <i>E. coli</i>	Watery diarrhoea	Salads, Cheese
• Enterohaemorrhagic <i>E. coli</i>	Bloody diarrhoea	Ground beef, salami
• Non-typhoidal salmonella	Inflammatory diarrhoea	meat, eggs, milk (unpasteurized)
• <i>Shigella</i>	Dysentery	Potato, egg salad
• <i>Vibrio parahaemolyticus</i>	Dysentery	raw or undercooked shellfish
• <i>Clostridium botulinum</i>	Flaccid paralysis, diplopia, dysphagia	Improperly canned food
• <i>Cyclospora</i>	Watery diarrhoea	Raw fruits & vegetables

***Clostridium perfringens*:** results from survival of heat-resistant spores in inadequately cooked beef meat, poultry, legumes

→ illness is self-limited & lasts for < 24 hrs.

→ mainly causes skin & soft-tissue infections.

***Listeria*:** usually affect pregnant women

→ fever & other flu-like symptoms (elderly people may develop meningitis)

→ soft cheese, raw sprouts, meat, raw milk

→ infections during pregnancy can lead to serious meningitis & sepsis in newborns

***Norovirus*:** source - contaminated salad, fresh fruits, shellfish

Mycotic poisoning: rare; caused by fungi.

Laboratory Diagnosis:

→ Ideal specimens: vomitus, stool, suspected food source

Processing of Food Specimens:

• **Viable Plate Count (Direct Quantification) / Standard Plate Count:**

10g of food material → sterile container → homogenized in 90 mL of sterile diluent → serial dilutions of homogenate or diluent is made → plated onto proper medium

[For intact vegetable/fruit, take 100g food sample in a sterile container containing 100mL of sterile water]

• **Pre-enrichment Culture:** pathogenic bacteria from suspected food sample is isolated by agar culture after pre-enrichment, followed by selective enrichment promoting the growth of target organism.

Toxin Detection:

• ELISA - for detection of enterotoxins in stool

• PCR - for detection of genes coding for enterotoxins

Staphylococcal Food Poisoning: Staph. produces enterotoxin (a preformed toxin) ∴ IP = 1-6 hrs.

→ toxin stimulates the vagus nerve & vomiting centre of the brain; also stimulates intestinal peristaltic activity.

→ Symptoms: nausea, vomiting, diarrhoea (occasionally), hypotension, dehydration, no fever
↳ resolve within 8-10 hours

→ Common food sources: ham, poultry, dairy products

→ staphylococcal enterotoxin is a heat stable toxin & is resistant to gastric juice

→ Enterotoxins ⇒ 15 serotypes (A-E, G-P)

• Type A is the most common cause of food poisoning

• Serotype F does not cause food poisoning; it causes toxic shock syndrome.

→ Detection of enterotoxin in food by ELISA or latex agglutination

→ Tx: supportive; by correcting fluid & electrolyte imbalance

***Bacillus cereus* Infection:** *B. cereus* is a normal habitant of soil; sources - vegetables, milk, cereals

↳ mediated by producing two types of toxins - diarrhoeal toxin & emetic toxin.

<i>Bacillus cereus</i>	Diarrhoeal type	Emetic Type
Incubation period	8-16 hrs	1-5 hrs
Toxin	Secreted in intestine	Preformed toxin
Heat	Heat labile	Heat stable
Food Sources	Meat, vegetables, dried beans	Chinese fried rice
Clinical Features	Diarrhoea, fever, abdominal cramps	vomiting, abdominal cramps
Serotypes involved	2, 6, 8, 9, 10, 12	1, 3, 5

→ Other manifestations of *B. cereus*:

• **Ocular disease:** severe keratitis, panophthalmitis, may lead to loss of vision.

• **Systemic infections:** endocarditis, meningitis, osteomyelitis, pneumonia.

Laboratory Diagnosis: *B. cereus* is isolated from faeces by using selective media such as MYPA (mannitol, egg yolk, polymyxin B, phenol red & agar)

→ gram +ve, spore-bearing motile bacillus

Treatment: *B. cereus* is susceptible to clindamycin, erythromycin, vancomycin, tetracycline

→ resistant to penicillin (by producing β -lactamase) & trimethoprim.

Food Poisoning

Treatment: adequate rehydration & electrolyte supplementation

• **Absorbent:** aluminium hydroxide

• **Antisecretory agent:** bismuth & subsalicylate

• **Antitoxin:** heptavalent botulism equine serum antitoxin may be given

• **Diet:** during episodes of acute diarrhoea, patients often develop acquired disaccharidase deficiency due to wash-out of brush-border enzymes ∴ avoid milk, dairy products & other lactose-containing food.

• **Antibiotics:** shigellosis - fluoroquinolone
suspected cholera - azithromycin.

Botulism:

Clostridium botulinum: - gram positive

- spore forming - obligate anaerobe - bacilli
- produces botulinum toxin - ubiquitous in nature
- non-invasive

Pathogenesis: Botulinum toxin (BT) $\Rightarrow$ powerful neurotoxin

(probably the most toxic substance known to be lethal to mankind)

$\rightarrow$ 8 serotypes - A, B, C1, C2, D, E, F, G.

- serotypes A, B, E commonly cause human disease
- serotype A $\Rightarrow$ most severe
- All serotypes produce neurotoxin except C2 which produces enterotoxin
- BT types C & D are bacteriophage coded

$\rightarrow$ BT is produced intracellularly, not secreted & appears outside only after autolysis of bacterial cell.

$\rightarrow$ toxin is synthesized initially as nontoxic prototoxin; it requires trypsin or other proteolytic enzymes to convert it into active form.

Mechanism of Action: BT produces flaccid paralysis

$\hookrightarrow$ BT is transported via blood to peripheral cholinergic nerve terminals

$\rightarrow$ site of action $\Rightarrow$ Neuromuscular junction (NMJ) (does not affect the CNS)

$\rightarrow$ BT binds to ACh receptors on the nerve terminals causing blockade of release of ACh leading to flaccid paralysis.

Therapeutic Uses: for treatment of spasmotic conditions such as strabismus, blepharospasm, myoclonus

Recovery: blocking of ACh release is permanent but recovery occurs within 2-4 months

$\rightarrow$ spores do not produce BT; toxin production requires spore germination in anaerobic atmosphere.

$\rightarrow$ spores do not normally germinate in adult intestine (they may germinate in the intestine of infants)

Clinical Manifestations: incubation period = 18-36 hours

- diplopia, dysphagia, dysarthria
- descending symmetric flaccid paralysis of voluntary muscles
- $\downarrow$ deep tendon reflexes
- constipation
- no sensory or cognitive deficits.
- respiratory muscle paralysis may lead to death.

Types of Botulism:

• **Food-borne botulism**: from homemade improperly canned or fermented food & beverage

$\rightarrow$ most cases are sporadic; outbreaks are rare

• **Wound botulism**: contamination of wounds with C. Botulinum spores

$\rightarrow$ absence of G-I features

$\rightarrow$ recurrent in injection drug users

• **Infant botulism**: most common type

$\hookrightarrow$ results from ingestion of contaminated food (usually honey) with spores of C. botulinum in children $\leq$ 1 year of age

$\rightarrow$ Manifestations: inability to suck & swallow, weakened voice, ptosis, floppy neck, extreme weakness $\Rightarrow$ **Floppy Child Syndrome**.

$\rightarrow$ self-limiting; rarely progresses to generalized flaccidity, respiratory failure & sudden death.

• **Adult Intestinal botulism**: in patients with suppressed normal flora, colonised clostridial spores may germinate to produce toxin.

• **Iatrogenic botulism**: results from injection of overdose of the toxin when used for therapeutic purpose.

• **Inhalational botulism**: aerosolization of spores in acts of bioterrorism.

Laboratory Diagnosis: isolation of bacilli, demonstration of toxin

• **Isolation of Bacilli**: gram-staining smears made from food or faeces - gram positive, non-capsulated bacilli with subterminal oval bulging spores.

$\rightarrow$ motile by peritrichate flagella

$\rightarrow$ culture is done on blood agar or Robertson's cooked meat (RCM) broth; they are either proteolytic or saccharolytic turning the meat particles into black & pink respectively.

$\rightarrow$ automated method = MALDI-TOF

• **Toxin Demonstration**: specimens - serum, stool, wound material, samples of ingested food.

$\rightarrow$ specimens are injected into mouse that develops paralysis in 48 hours (this paralysis can be inhibited by prior administration of specific antitoxin)

Treatment:

Botulinum Antitoxin: administered immediately on clinical suspicion without waiting for laboratory confirmation

(once toxin binds to the nerve endings, antitoxin is of no use)

$\rightarrow$ in wound botulism, suspected wounds & abscesses should be cleaned, debrided & drained promptly.

• Antibiotics: penicillin or metronidazole

Mycotoxicoses:

Mycotic Poisoning:

Mycotoxicosis: disease produced by consumption of food contaminated with toxins liberated by fungi.

Mycetism: toxic effects produced by eating poisonous fleshy fungi (usually mushrooms).

Mycotoxicosis:

Mycotoxin	Fungi
Aflatoxin	Aspergillus sp.
Fumonisin	Fusarium moniliforme
Trichothecenes	Fusarium graminearum
Ochratoxin	Aspergillus
Cyclopirolic acid	Penicillium cyclopium
Zearalenone	Fusarium graminearum

Mycetism:

Mushroom Poisoning	Fungi	Clinical Condition
Ergot alkaloid	Claviceps purpurea	St. Anthony's fire
Coprine poisoning	Coprine atramentarius	Antabuse-like reaction
Muscarine	Inocybe fastigiata	Cholinergic effect
Ibotenic acid, muscimol	Amanita pantherina	Abdominal pain, vomiting, diarrhoea
Cyclopeptide	Amanita phalloides	Hepatocellular failure, green death cap

Chemical Causes of Food Poisoning: (non-microbial agents)

- capsaicin (found in hot peppers)
- toxins found in fish & shellfish
- ciguatera
- histamine-fish poisoning (scombrotoxin)
- heavy metals

Vibrios: gram negative bacilli

- actively motile, single polar flagellum (vibratory motility)
- characteristic curved or comma-shaped appearance
- ubiquitous in nature
- salt-loving (halophilic)
- *V. cholerae* ⇒ acute diarrhoeal disease: Cholera.

Classification of Vibrios:

I Based on Salt Requirement:

- growth of vibrios is stimulated in presence of salt.
- **Nonhalophilic Vibrios**: can grow without salt.
 - 1% salt is optimum for their growth (cannot grow at higher salt concentrations)
 - *V. cholerae* – *V. mimicus*
- **Halophilic vibrios**: cannot grow in absence of salt
 - can grow at higher salt conc. of upto 7-10%
 - *V. parahaemolyticus* – *V. alginolyticus*
 - *V. vulnificus*

II Gardner & Venkatraman Classification: based on serogrouping, biotyping, serotyping & phage typing.

- **Serogrouping**: Based on somatic O antigen (Sakazaki typing scheme)

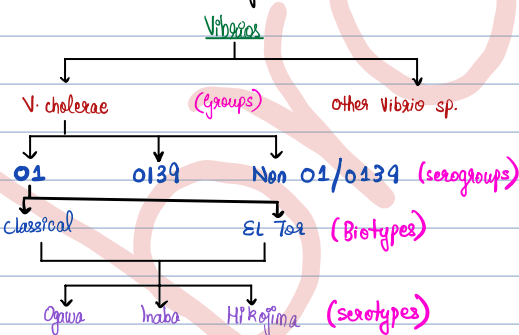
O1 serogroup: responsible for all pandemics & most of the epidemics of cholera.

NAG Vibrios: not agglutinated by O1 antiserum (non-agglutinable ⇒ NAG)

→ were thought to be non-pathogenic ∴ previously called non-cholera (NCV) vibrios

O139 serogroup: caused several epidemics

Non O1/O139 serogroup: sporadic outbreaks



Biotyping: based on susceptibility to polymyxin B

Classical Type: responsible for first 6 pandemics of cholera.

ET Tox Type: caused 7th pandemic of cholera

Serotyping:

Ogawa: most common serotype

Inaba: due to mutation in rfbT gene

Hikojima: unstable transitional state where both Inaba & Ogawa antigens are expressed

Cholera & Vibrio

Laboratory Diagnosis:

- **Specimens**: watery stool (rectal swab for carriers)
- **Transport Media**: VR medium, Cary-Blair Medium
- **Direct Microscopy**: gram negative rods, short curved comma-shaped (fish-in-stream appearance)
 - hanging drop demonstrates darting motility.
- **Culture**: **Enrichment broth**: Alkaline peptone water, Monawa's tauracholate tellurite peptone water.
- Selective Media**: Bile Salt Agar, Monawa's GTT agar, TCBS agar (yellow colonies)
- MacConkey Agar** produces translucent NLF colonies.
- **Culture smear**: short curved gram negative bacilli.

Pathogenesis: toxin-mediated

- O1 & O139 are capable of toxin production
- transmitted by ingestion of contaminated water/food
- infective dose: 10^8 bacilli are required to bypass the gastric barrier
- factors promoting transmission: conditions where gastric acidity is reduced (hypochlorhydria, use of antacid, etc.)
- in small intestine, vibrios penetrate the mucus layer & reach near the epithelial cells by:
 - highly active motility
 - secreting mucinase & other proteolytic enzymes
 - secreting haemagglutinin protease (cholera lectin) which cleaves mucus & fibronectin.

→ vibrios then adhere to the intestinal epithelium which is mediated by a special type IV fimbria called Toxin-Coregulated Pilus (TCP).

- once established in the small intestine, they produce powerful enterotoxin - Cholera Toxin [CT]
- CT resembles heat-labile toxin (LT) of *E. coli* in its structure & function, but it is more potent than LT.

Mechanism of Action of CT: CT consists of 2 peptide fragments - A & B

Fragment B: [Binding Fragment] binds to GM1 ganglioside receptors on intestinal epithelium following which fragment A is internalised.

Fragment A: [Active Fragment] causes ADP ribosylation of G-protein → upregulates activity of adenylate cyclase → results in intracellular accumulation of cAMP.

Increased intracellular cAMP leads to:

- in small intestine - inhibits the absorptive sodium transport in villus cells & activates the secretory chloride transport in crypt cells ⇒ accumulation of NaCl in intestinal lumen.
- water passively moves into bowel lumen to maintain osmolality which leads to watery diarrhoea.
- loss of fluid & electrolytes leads to shock (due to dehydration) & acidosis (due to loss of bicarbonates)

Gene for CT (CTX): CT is phage coded [encoded by the genome of a filamentous bacteriophage (CTX)]

→ other virulence factors include:

- **Zona occludens toxin**: disrupts the tight junctions between mucosal cells
- **Accessory colonization factors**: help in adhesion & colonization
- **Bacterial endotoxin (LPS)**: unlike other gram -ve bacilli, LPS of *V. cholerae* does not contribute to the pathogenesis of cholera; but it is immunogenic & thus, included as a component in killed vaccines.

Identification:

- catalase & oxidase positive
- ICUT: indole (+), citrate (+/-), urease (-), TSI: A/A, gas (-), H_2S (-)
- string test positive
- produces hemolysis on blood agar
- automated: MALDI-TOF, VITEK
- **Biotyping**: to differentiate classical & ET Tox
- **Antigen Detection**: by cholera dipstick assay
- **Molecular method**: multiplex PCR detecting common diarrhoeal pathogens
- **Antimicrobial susceptibility testing** (on Mueller-Hinton Agar)

Clinical Manifestations:

- asymptomatic infection (75% cases)
- mild diarrhoea/cholera (20% cases)
- sudden onset of explosive & life-threatening diarrhoea (5% cases)
- incubation period: 24-48 hrs.
- Watery diarrhoea**: sudden onset of painless watery diarrhoea that may become voluminous.
- Rice-water stools**: stools - non-bilious, slightly cloudy & watery with mucus flakes, fishy offensive odour
 - no associated blood or pus cells in stool
- Vomiting**: fever is usually absent
- Muscle cramps** may occur due to electrolyte imbalance
- Progression of clinical manifestations** is directly proportional to fluid loss resulting in loss of body weight.

Epidemiology:

- Homeland of cholera was: Delta region of Ganges & Brahmaputra in West Bengal & Bangladesh
- First 6 pandemics: 1817 to 1923 (started from Bengal)
- 7th pandemic started in 1961 in Indonesia & was caused by the **ET Tox** biotype
- Serogroup O139 was known as Bengal strain ∴ it spread rapidly along the coastal region of Bay of Bengal upto West Bengal.
- **Ending cholera: A Roadmap to 2030**
 - launched in 2017 by WHO
 - aims to reduce cholera deaths by 90% & to eliminate cholera in 20 countries by 2030.
- **NICED**: National reference Center for Cholera in India is located in National Institute of Cholera & Enteric Diseases, Kolkata.
- humans are the only reservoir of infection
- source of infection: asymptomatic cases or carriers
- biotype ET Tox has more carriers rate than classical.
- Patients when effectively treated do not become carriers after they recover, but can be reinfected if exposed again.
- maximum transmission is associated with high temperatures, heavy rainfall & flooding.
- other factors that promote transmission: poor sanitation, poverty, overcrowding, population mobility, etc.
- **Factors determining severity of the disease**:
 - **Persons with blood group O** are at a greater risk of severe disease (those with blood group AB are at least risk)
 - Malnutrition
 - Immunocompromised individuals
- **Age**: during inter-epidemic period: all age groups during epidemics: children more frequently
- *V. cholerae* is a natural inhabitant of coastal sea salt water & brackish estuaries.

Resistance:

- *V. cholerae* is acid-labile but stable to alkali
- Heat-labile (killed within 30 minutes by heating at 56°C or within few seconds by boiling), but stable to refrigeration & can remain in ice for 4-6 weeks.
- Drying & sunshine can kill the bacilli in a few hours
- susceptible to disinfectants such as cresol & bleaching powder (6 mg/L)
- biotype ET Tox is more resistant than classical.

→ If delay is expected, stool (1-3 ml) or rectal swabs may be inoculated in 10-20 ml of:

- Venkatraman-Ramakrishnan (VR) medium

- Alkaline salt transport medium
- Cary Blair medium
- Autoclaved sea water

→ fecal specimen is inoculated onto enrichment broth & incubated for 4-6 hrs following which a subculture is made onto another selective medium - Alkaline Peptone Water (APW)

- Monsur's tauracholate tellurite peptone water.

→ stool sample is directly inoculated onto a selective media & incubated for 24 hrs at 37°C

- *V. cholerae* produces translucent oil drop colonies in alkaline BSA.

- In Monsur's GTTA, *V. cholerae* produces translucent colonies with a greyish black center & turbid halo

→ On blood agar, it causes nonspecific lysis, seen as a greenish clearing around the main inoculum.

→ When a colony of *Vibrio* is mixed with a drop of 0.5% sodium deoxycholate on a slide, the suspension loses its turbidity & becomes mucoid. When tried lifting with a loop, it forms a string

Treatment:

Fluid Replacement: prompt & adequate to correct hypovolemia & maintain water levels in the ongoing fluid loss.

- In mild/moderate fluid loss: ORS
- In severe cases: IV replacement till the patient gains consciousness following which ORS can be used

Antibiotics: not necessary; may be used to decrease the duration of fluid loss & preventing carrier stage.

- **DOC:** Macrolides

(Alternative: doxycycline/ciprofloxacin)

Prevention:

General Measures:

- provision of safe water
- improved sanitary disposal of faeces
- proper food sanitation, hand hygiene
- prompt outbreak investigation

Cholera is a notifiable disease locally & nationally.

Chemoprophylaxis: **DOC** = tetracycline (Alternative = doxycycline)

- indicated to household contacts
- mass antibiotic prophylaxis leads to development of resistance & is thus, not recommended by WHO.

Vaccines:

- **Injectable Killed Vaccines:** no longer used.
 - offers little protection
 - causes adverse effects & fail to induce local intestinal mucosal immune response

Oral Cholera Vaccines:

i. Killed whole-cell vaccine

Whole Cell (WC) vaccine

- composed of killed whole cells of 01 & 0139
- two formulations - Shanchol (India) & Evichol (South Korea)
- 2 doses with minimum gap of 2 weeks (>1 years of age) (orally)
- provides protection against cholera for 3 years

WC recombinant B subunit vaccine:

- WC + cholera toxin B subunit
- available commercially as Dukoral
- 2 doses given orally with a minimum gap of 1 week
- 3rd dose for children of 2-5 years (>2 years of age)
- provides protection against cholera for 2 years.

ii. Oral Live Attenuated Vaccine (OCV):

- uses mutant strains that lack the gene encoding for cholera toxin

CVD 103-HgR: commercially available as Vaxchora

- single oral dose
- recommended for adults [18-64 years] travelling to an area with active cholera transmission
- gives 90% protection 10 days after vaccination
- protection lasts for 3-6 months.

Halophilic Vibrio Infections:

→ halophilic vibrio can withstand higher salt conc. (>6%) in contrast to *V. cholerae*.

eg: *V. parahaemolyticus*

Clinical Manifestations: food-borne gastro-enteritis

- watery diarrhoea
- dysentery with abdominal cramps (rare)
- extraintestinal manifestations - wound infection, otitis, sepsis

Laboratory Diagnosis: similar to that of *V. cholerae*

- capsulated, shows bipolar staining in fresh isolates & pleomorphism in older culture
- motile by peritrichous flagella
- green colonies on TCBS (non-sucrose fermenter)
- **Kanagawa Phenomenon:** it causes β -hemolysis on Wagatsuma agar (high salt blood agar)
- it swarms on blood agar
- urease test +ve in few strains
- it can resist maximum of 8% NaCl.

Treatment:

- most of the gastroenteritis is self-limiting & treatment is same as cholera
- indications for antibiotic use: - severe gastroenteritis, extraintestinal manifestations associated with underlying disease.
- **DOC:** Doxycycline or macrolide

***Vibrio Mutilans* Infections:** rare but most severe among the infections caused by *Vibrio* species.

Clinical Manifestations:

Primary sepsis: usually occurs in patients with underlying liver diseases, iron overload, renal insufficiency, immunosuppression.

Primary wound infection: characterized by painful erythematous swelling or cellulitis or even vesicular, bullous, necrotic lesions; generally affect people without underlying diseases.

Laboratory Diagnosis: culture from blood or cutaneous lesions

- it ferments lactose which differentiates it from other vibrios.

Treatment: early antibiotic institution, wound debridement & general supportive care

→ drugs: tetracycline, fluoroquinolones, third-generation cephalosporins

***Vibrio Alginolyticus* Infections:** occasional eye, ear & wound infections

→ otitis externa, otitis media, conjunctivitis

→ it rarely causes bacteremia in immunocompromised.

→ most salt-tolerant *Vibrio* (>10%)

→ disease is self-limiting

→ in case of severe infections: • tetracycline • drainage

Aeromonas Infections:

→ *Aeromonas* was earlier placed in family Vibrionaceae

→ later, it was put into a separate family - Aeromonadaceae

eg: *A. hydrophila* → causes red leg disease in frog

Pathogenesis:

→ tissue adherence is mediated by adhesins such as S-layer & fimbriae

→ Capsular polysaccharide (prevents phagocytosis of bacilli)

→ exotoxins such as aerolysin, phospholipases, hemolysin, enterotoxins & cytotoxin similar to Shiga toxin

→ Endotoxin or LPS.

Clinical Manifestations:

- Gastroenteritis (watery diarrhoea, vomiting, fever, rarely dysentery)

- peritonitis

- musculoskeletal & wound infections

- bacteremia in immunocompromised adults & infants

- respiratory tract infections (epiglottitis, pharyngitis, pneumonia)

Laboratory Diagnosis: automated identification systems such as MALDI-TOF & VITEK or various conventional biochemical tests.

Treatment: *Aeromonas* is susceptible to ciprofloxacin & levofloxacin

→ alternatives: cotrimoxazole, ceftiofime

→ plasmid-induced drug resistance has been reported including β -lactamase production.

Viral Gastroenteritis

Rotavirus Diarrhoea

→ Rotavirus are the most common cause of diarrhoea in children

Morphology:

- Icosahedral symmetry
- triple-layered wheel-shaped capsid
- segmented dsRNA (11 segments)
- **Proteins**: 6 structural viral proteins (VP1-VP7 except VP5)
- 6 non-structural proteins (NSP1-6)
- VP7 forms outer capsid layer; VP4 forms spikes that emanate through the outer capsid layer

Virus Causing Gastroenteritis:

Virus	Genome
Rotavirus	segmented dsRNA
Caliciviruses	
- Norovirus	ssRNA
- Sapovirus	ssRNA
Astrovirus	ssRNA
Adenovirus	dsDNA

Classification of Rotaviruses:

- **Traditional Groups**: seven major groups (A-G)
- most human diarrhoea is caused by group A
- **Binary System**: combination of both serotyping & genotyping
- Two groups: 19G & 28[P]
- most common type: G1P[8].

Pathogenesis:

→ Transmission: by feco-oral route

↓
destruction of enterocytes of small intestine [gastric & colonic mucosa are spared]

↓
multiplication in cytoplasm of enterocytes & damage their transport mechanisms resulting in secretory diarrhoea

↓
NSP-4 acts as enterotoxin & induces secretion by altering epithelial cell function & permeability

Clinical Manifestations:

- incubation period: 1-3 days
- abrupt onset
- vomiting, watery diarrhoea, fever & abdominal pain.

Epidemiology:

- Group B rotaviruses: outbreaks of severe gastroenteritis in adults in China.
- In developing countries like India, rotavirus illness occurs at a younger age

Laboratory Diagnosis:

- **Direct Detection of Virus**: immunoelectron microscopy (IEM) show rotavirus having sharp-edged triple shelled capsid [spokes grouped around the hub of a wheel]

- **Detection of Viral Antigens**: in stool by ELISA & latex-agglutination methods
- **RT-PCR**: for detection of rotavirus in stool
- **Typing methods**: detection of G serotype by RNA sequence typing; detection of P genotype by neutralisation test.
- **Serologic Tests (ELISA)**: for detection of rise of antibody titre

Treatment: mainly supportive - to correct loss of water & electrolytes using ORS or IV fluids

Prevention:

- **Rotavac**: contains live attenuated rotavirus 116E {G9P[15]}
 - ↳ manufactured by Bharat Biotech, India
 - ↳ 3 doses: 5 drops/dose, oral, at 6, 10, & 14 weeks along with DPT & OPV
 - side effects: crying, irritability, fever, diarrhoea.
- **Rotarix**: live attenuated G1P[8] strain
 - ↳ has to be reconstituted before use
 - 2 doses: 6th week, 10th week

General Preventive Measures:

- Improving hygiene & sanitation in the community
- Contact precautions (like hand hygiene) to prevent person-to-person transmission

Family Caliciviridae Gastroenteritis:

- Family Caliciviridae has 4 genera
- 2 important diarrhoeal genera - Norovirus & Sapovirus

- Icosahedral symmetry
- cup-like depressions on the capsid surface (as seen on electron microscopy)
- ssRNA(+) with one single major structural protein

Norwalk Virus: most important cause of epidemic viral gastroenteritis in adults

- common in winter months ∴ called 'winter vomiting disease' or 'gastric flu'
- symptoms begin 12-48 hours after exposure
- Common food sources: fresh fruits, shellfish or water

Adenovirus (types 40 & 41): second most common cause of viral gastroenteritis of infants & young children

Astroviruses: distinctive star-like morphology

- Icosahedral symmetry, (+) ssRNA.

Respiratory viruses:

- SARS coronavirus
- Influenza A/H1N1 virus
- Influenza A/H5N1 virus

Toroviruses & Picobirnaviruses: cause gastroenteritis in a variety of animals.

Intestinal Amoebiasis

Intestinal amoebae - *Entamoeba histolytica*

Free-living amoebae - *Naegleria*, *Acanthamoeba*, *Balamuthia*

Entamoeba histolytica

- causes amoebic dysentery
- *E. dispar*, *E. moshkovskii*, *E. bangladeshi* are morphologically similar to *E. histolytica*
- all of them are commensals in human intestine
- *E. moshkovskii*, *E. bangladeshi* are associated with infantile diarrhoea.

Epidemiology: worldwide; more common in tropical & subtropical countries.

→ 3rd most common parasitic cause of death in the world (after malaria & schistosomiasis)

Morphology: *E. histolytica* exists in 3 stages

- **Trophozoite:** invasive form
 - found in the feces of patients with active disease
 - possesses finger-like projections called pseudopodia which help in locomotion.
- **Pre-cyst:** intermediate stage
- **Cyst:** diagnostic form
 - found in the feces of carriers & patients with active disease
 - can be mature or immature
 - [immature have 1/2 nuclei;
 - mature forms are quadrinucleated & are the infective form]

Life Cycle:

- **Host:** single host - man
- **Infective form:** mature quadrinucleated cysts (immature cysts too)
- **Mode of transmission:** feco-oral route (most common), sexual (anogenital or orogenital), rarely by vectors (flies & cockroaches)

Cyst bypasses gastric juice in stomach to reach small intestine

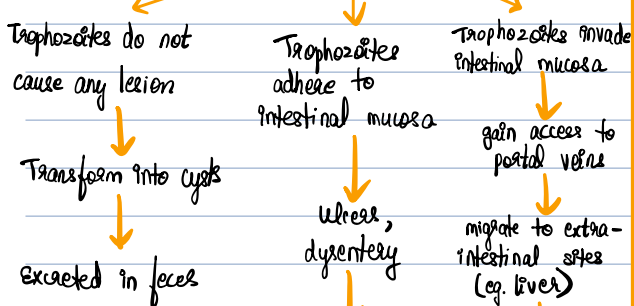
Excystation: lysis of cyst wall with trypsin

Release of 4 small trophozoites

Trophozoites are carried to ileo-caecal junction of large intestine

multiplication by binary fission

colonization of intestinal mucosa



Trophozoites are also excreted but get dis-integrated in the environment or by gastric juice when ingested.

Virulence Factors:

- **Amoebic lectin antigen:** surface protein [gal/NAG lectin]
 - helps in adhesion by binding to glycoprotein receptors on large intestinal epithelium & vascular endothelium.
- **Amoebapore:** it forms pores on target cell membrane causing leakage of ions
- **Cysteine proteases & hydrolytic enzymes:** help in invasion by the destruction of target tissue
- **Neuraminidase & metallocollagenase:** help in invasion

Pathogenesis: Steps

- **Colonization:** trophozoites colonize intestinal mucosa & this is facilitated by bacterial flora which lower the oxygen tension.
- **Adhesion:** trophozoites penetrate the mucus layer & adhere to large intestinal mucosa by gal/galNAc lectin molecules
- **Flask-shaped ulcers:** trophozoites produce characteristic flask-shaped ulcerative lesions (broad base with narrow neck) in large intestine
- **Invasion:** amoebae invade large intestinal wall & migrate to extraintestinal sites (facilitated by cysteine proteases & hydrolytic enzymes)
- **Intestinal complications:** Fulminant Amoebic Colitis → generalized necrotic involvement of entire large intestine.

Laboratory Diagnosis:

- **Stool microscopy:**
 - **Stool sample:** minimum 3 stool samples collected on alternate days (within 10 days)
 - **Macroscopy:** foul smelling, copious in amount, dark red in colour mixed with blood & mucus, not adherent to the container
 - **Microscopy:** [Direct examination of stool by saline & iodine mount]
 - cysts & trophozoites of *E. histolytica* are indistinguishable from that of *E. dispar* or *E. moshkovskii* or *E. bangladeshi*, except for the presence of RBCs in trophozoites of *E. histolytica*

Trophozoites: demonstration of trophozoites in freshly passed stool ⇒ Gold Standard for active infection

- **cytoplasm** is divided into a clear ectoplasm & a granular endoplasm; single nucleus.
- actively **motile** (with finger-like pseudopodia)
- **hematophagous trophozoite:** presence of ingested RBCs in cytoplasm
- **Nucleus:** has a central karyosome & fine peripheral chromatin granules [Coat wheel appearance]
- trophozoites are better-appreciated in saline mount than iodine mount.

Quadrinucleated Cysts: cysts are found in stool specimens of carriers & patients with active infection

- 1-4 nuclei
- mature cysts (4 nuclei)
- immature cysts (1-2 nuclei)
- **Histology:** trophozoites can be detected in sigmoidoscopy guided biopsies stained with PAS or H&E stains, or ideally with peroxidase staining with anti-*E. histolytica* antibodies.

• **Stool Culture:** Various culture media -

→ **Polyxenic culture:** contains bacterial supplement, starch, serum

• National Institute of Health (NIH) media

• Boeck & Drbohlav egg serum medium containing Locke's solution.

→ **Axenic Culture:** lacks bacterial supplement eg. Diamond's medium

• **Stool Antigen Detection [Coproantigen]:** amoebic antigens get denaturated by stool preservatives, only fresh or frozen stool sample should be used.

→ ELISA

→ Immunochromatographic test (ICT)

• **Serology:**

→ **Amoebic Antigen:** found in serum of only patients with active infection

→ detection of lectin antigen by ELISA.

→ **Amoebic Antibody:** detecting serum antibody (IgG) against lectin antigen by ELISA.

→ **Isoenzyme (zymodeme) Analysis:** when isoenzymes of *E. histolytica* are subjected to electrophoresis, several zymodeme patterns can be produced

→ it can differentiate *E. histolytica* from *E. dispar*

• **Molecular Diagnosis:** Gold Standard for diagnosis of Amoebiasis

→ Nested multiplex PCR targeting small subunit rRNA gene

→ Real-time PCR targeting 18S rRNA gene

→ BioFire FilmArray (fully automated commercial PCR system)

• **Non-specific Findings:**

→ Colonoscopy: detects collar button or flask-shaped amoebic ulcers

→ Charcot-Leyden crystals in stool: diamond shaped, eosinophilic breakdown products

→ Moderate leukocytosis in blood.

Treatment:

• **Asymptomatic Carriage:** treatment to help in reducing passage of cysts in stools & ∴ preventing further transmission

→ Use of luminal agents for Tx:

→ Iodoquinol (for 20 days) or

→ Paromomycin (for 10 days)

→ **Amoebic Dysentery:**

→ Luminal agents as above

→ Tissue agents: - Metronidazole (5-10 days) or

- Tinidazole (3 days)

→ fluid & electrolyte replacement.

Prevention: avoid ingestion of food & water contaminated with human feces & treatment of asymptomatic carriers

Amoebae Morphologically Resembling *E. histolytica*:

→ *E. dispar*, *E. moshkovskii*, *E. bangladeshi*

• *E. dispar*: non-pathogenic commensal found in human intestine

• *E. moshkovskii*: found in polluted water

• *E. bangladeshi*

Diagnosis: they can be differentiated from *E. histolytica* by:

→ PCR amplifying small subunit rRNA gene

→ detection of lectin antigen in stool

→ RBC inside trophozoites (found only in *E. histolytica*)

→ zymodeme study

Nonpathogenic Amoebae:

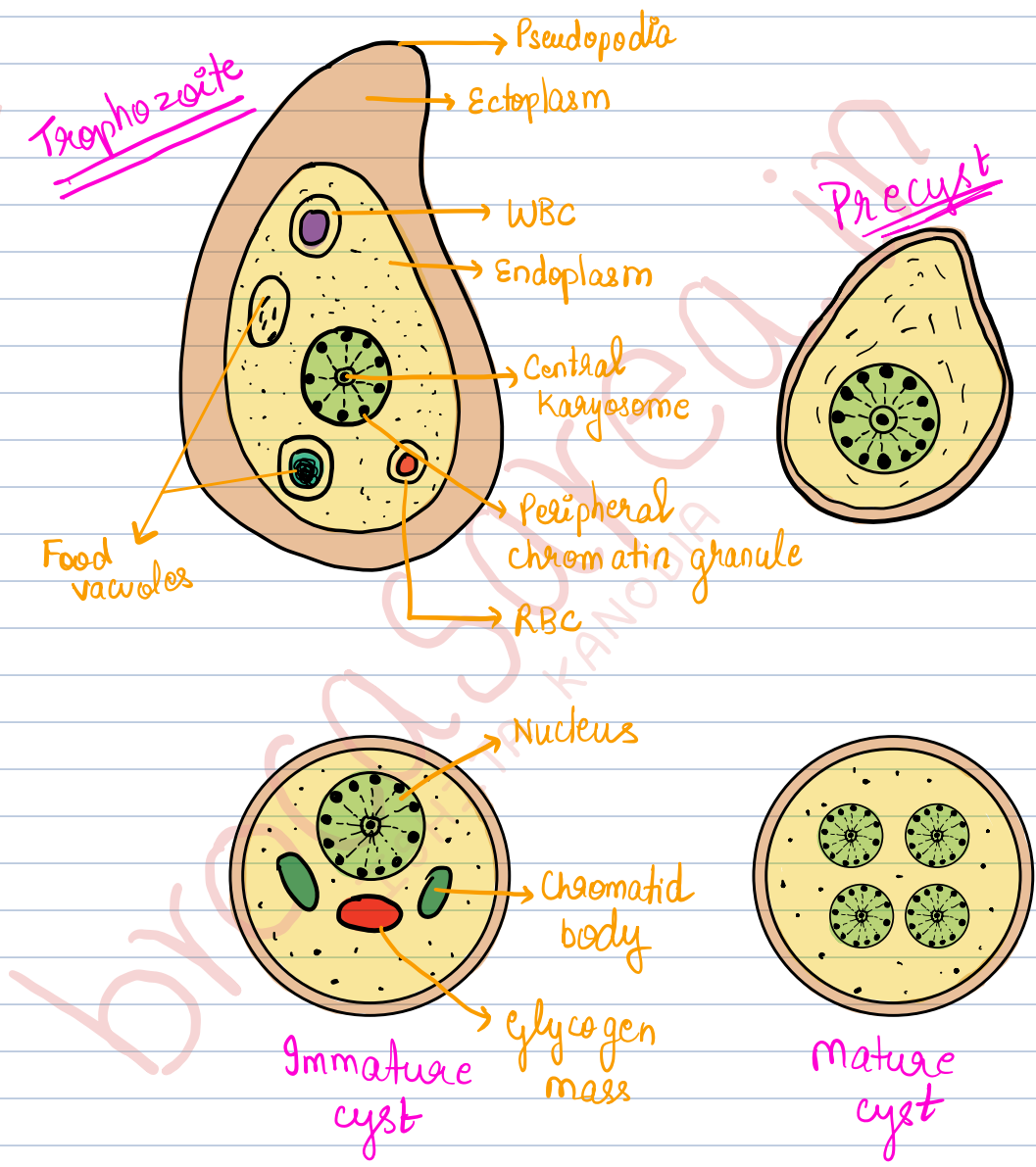
• *Entamoeba coli*

• *E. hartmanni* • *E. gingivalis* • *E. polecki*

• *Endolimax nana* • *Iodamoeba butschlii*

→ *Entamoeba coli*: colonizes the large intestine

Character	Amoebic Dysentery	Bacillary Dysentery
Ulcer	Deep	Shallow
Margin	Ragged & Undermined	Uniform
Intervening mucosa	Normal	Inflamed
Cellular response	Mononuclear	Polymorphonuclear
Number of motion	6 - 10 / day	> 10 / day
Amount	Copious amount	Small quantity
Colour	Dark red	Bright red
Odor	Offensive	Odorless
Reaction	Acidic	Alkaline
Consistency	Not adherent to container	Adherent to container
RBCs	In clumps	Discrete or in rouleaux
Pus cells	few	Numerous
macrophages	few	Numerous
Eosinophils	present	Absent / rare
Charcot - Leyden crystals	present	Absent
Pyknotic body	present	Absent
Organism detected	E. histolytica cyst or trophozoite	Bacteria (eg. Shigella)



Intestinal Flagellate Infections

Giardiasis

→ *Giardia lamblia* aka *G. intestinalis* or *G. duodenalis*

Epidemiology: more common in warm climate

→ *Giardia* has been classified into genotypes A & B which are further classified into 8 assemblages (A to H).

Morphology: inhabits in crypts of duodenum & upper part of jejunum in man

→ it occurs in 2 forms:

- **Pear-shaped trophozoite:** pathogenic form & feeding stage of parasite
- **Tetra-nucleated oval cyst:** infective form & diagnostic form

Life Cycle:

- **Host:** single host
- **Infective form:** cysts
- **Mode of transmission:** ingestion of food & water contaminated with cysts; sexual route (rarely)
- **Development in man:**
 - **Excystation:** release of 2 trophozoites from each cyst in duodenum within 30 minutes of entry.
 - **Multiplication:** by longitudinal binary fission in duodenum
 - **Adhesion:** trophozoites adhere to duodenal mucosa by the bilobed adhesive ventral disk.
[in active stage of infection, trophozoites are directly excreted in diarrhoeic stool due to sloughing of intestinal epithelial cells every 72 hours]
 - **Encystation:** begins when trophozoites pass down to jejunum.
 - it involves retraction of flagella into axoneme followed by formation of cyst wall
 - on maturation, nuclei divide to become four & the cysts are excreted in feces.

Pathogenicity:

- **Infective dose:** 10-25 cysts
- **Risk factors:** children, elderly debilitated persons, poor hygiene, immunodeficiency
- **Pathogenic form:** trophozoites
- **Malabsorption:** may be of various types
 - Malabsorption of fat (steatorrhea)
 - Disaccharidase deficiency
 - Malabsorption of vit. A, B12 & iron
 - Protein losing enteropathy
- **Antigenic variation:** *Giardia* undergoes frequent antigenic variations due to a cysteine rich protein on its surface called Variant Surface Protein (VSP)

Clinical Features:

 3 stages -

- **Asymptomatic Carriers:** asymptomatic but harbor the cysts in the gut & spread the infection
- **Acute giardiasis:** incubation period ⇒ 1-3 weeks
 - diarrhoea, abdominal pain, bloating, belching, flatus & vomiting
 - **Fatty diarrhoea (steatorrhea):** foul smelling with fat, cellular exudate, & mucus but no blood.

[*Giardia* is an important cause of traveler's diarrhoea]

- **Chronic giardiasis:** recurrent episodes of foul smelling diarrhoea, foul flatus, sulphurous belching with rotten egg taste & profound weight loss leading to growth retardation
- **Extraintestinal manifestations:** urticaria, arthritis, anterior uveitis, salt & pepper retinal changes

Laboratory Diagnosis:

- **Stool Examination:** stool microscopy ⇒ gold standard
 - trophozoites adhere firmly to duodenal mucosa by adhesive disk leading to intermittent shedding
∴ repeated stool examination should be done
 - pus cells or RBCs will never be seen in stool.
 - concentration techniques like zinc sulfate floatation or formalin ether sedimentation to increase chance of detection
- if stool examination is negative, then direct duodenal samples like aspirates/biopsy should be processed.
- permanent stains such as trichrome stain can be used to demonstrate cysts & trophozoites in stool.

Trophozoite: presence of trophozoites indicates active stage of disease

→ in front view: pear-shaped / tear-drop or tennis-racket shaped

→ laterally: spoon or sickle shaped

→ falling leaf-like motility

→ bilaterally symmetrical

- one pair of nuclei

- adhesive or suckling disk

- four pairs of flagella

- pair of axonemes

Cyst: oval-shaped; four nuclei & axonemes

→ cannot differentiate active disease & carriers.

- **Entero-test / String Test:** it uses a gelatin capsule attached to a thread containing a weight
 - one end of the thread is attached to outer aspect of patient's cheek & capsule is swallowed

→ capsule gets dissolved in stomach releasing the thread which reaches the duodenum, gets unfolded & takes up duodenal samples.

→ 4 hours later, thread is withdrawn & shaken in saline to release trophozoites which can be detected microscopically.

→ entero-test is also used for detection of *Strongyloides*, *Cryptosporidium*, *Clonorchis*

• **Histopathology:** endoscopy-guided duodenal biopsy tissue stained by Giemsa to demonstrate trophozoites.

• **Antigen Detection in Stool (Coproantigen):**

→ ELISA & direct fluorescent antibody tests using labeled monoclonal antibodies against cyst wall protein antigens.

→ Rapid ICT

• **Antibody Detection:**

→ indirect fluorescent antibody (IFA) & ELISA are used for detection of antibodies in serum.

• **Molecular Methods:** detection of *giardia* nucleic acid in stool & environmental samples by PCR or BioFire Film Array

• **Radiological Findings:** fluoroscopy may reveal hypermotility at the duodenal & jejunal levels
→ X-ray after barium meal ⇒ non-specific irregular mucosal thickening with large dilated loops of hypotonic bowel.

Treatment:

→ Tinidazole (2g once orally) ⇒ DOC

→ Metronidazole (5 days) or nitazoxanide (3 days) alternatively

→ Furazolidone for children & aurano-fin, paromomycin in pregnancy

→ In AIDS & hypogammaglobulinemia patients, giardiasis is often refractory to Tx. Use metronidazole for 21 days.

→ in case of metronidazole sensitivity, use aurano-fin.

Prevention:

→ improved food & personal hygiene.

→ boiling or filtering of potentially contaminated water.

→ Tx of asymptomatic carriers

→ no vaccine currently available.

Other Intestinal Flagellates of Minor Importance (harmless intestinal commensals of human GIT)

• *Trichomonas tenax* [mouth]

• *Pentatrichomonas hominis* (large intestine)

• *Chilomastix mesnili* (large intestine)

• *Enteromonas hominis* (large intestine)

• *Retortamonas intestinalis* (large intestine)

• *Dentamoeba fragilis* (large intestine)

