Cholera -Update

Dr Shabina MB
Additional Professor, Microbiology, GMC,Kozhikode

Cholera

- A highly contagious life threatening diarrheal disease affecting millions worldwide each year.
- Major public health problem, in countries with poor sanitary conditions affected by natural disasters, where access to safe drinking water is limited

Preventable and treatable disease, and several strategies can be used to control it

Safe drinking water, sanitation and hygiene (WASH)

Explosive epidemics

Pandemics

Vibrios -History

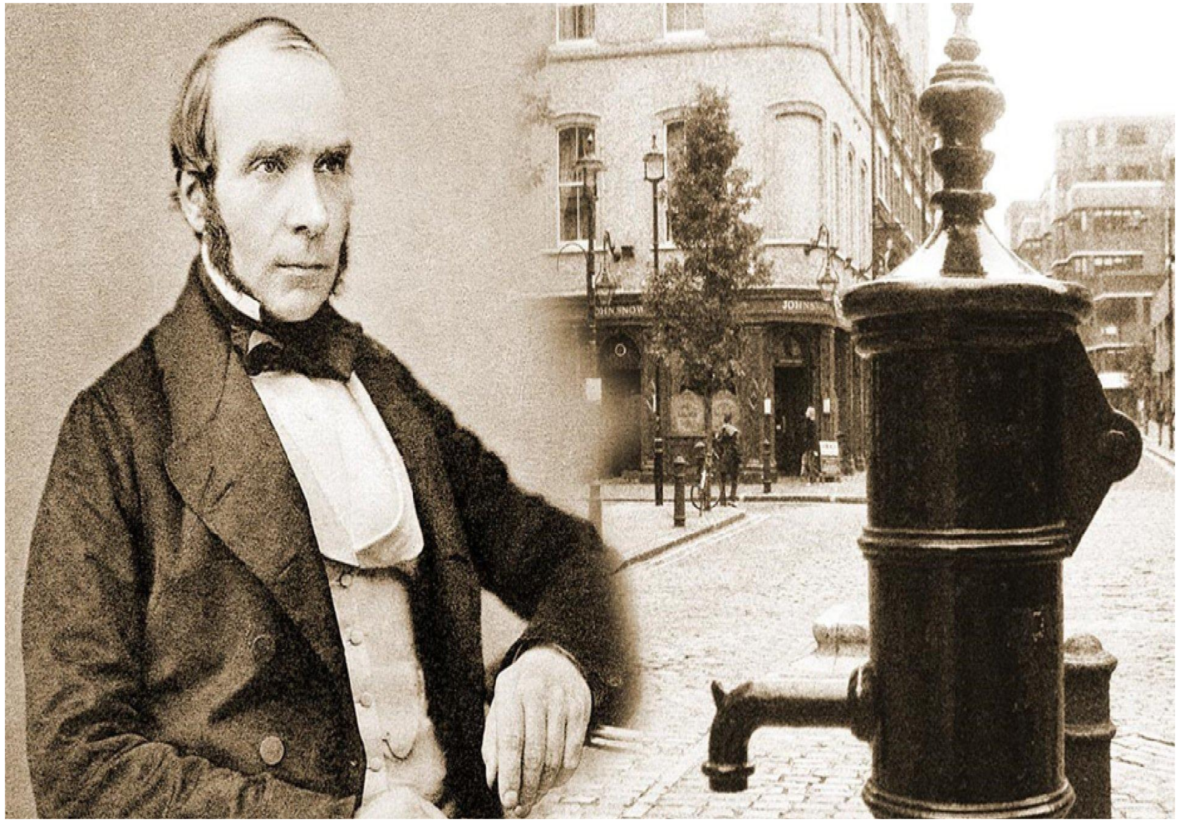
Curved gram-negative bacilli –Vibrio-vibratory motility

single polar flagellum.

Vibrio cholerae -Discovered by Filippo Pacini-1854

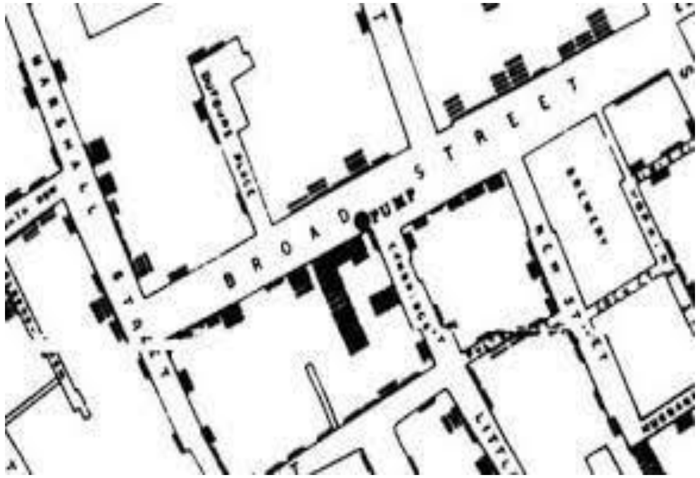
Robert Koch isolated the organism in 1886, Komma bacillus

John
Snow



56 deaths in 2 days

Role of the water supply in the Soho epidemic of 1854



Learning objectives

- Classification
- Pathogenesis, clinical manifestations
- Lab diagnosis and treatment and prevention

Recent outbreaks -WHO

- From 1 January 2023 to 31 March 2024 - total 824 479 cholera cases and 5900 deaths were reported globally across five WHO regions.

- Afghanistan , Democratic Republic of the Congo , Zimbabwe , Zambia and Somalia

- The world has run out of cholera vaccines

World Health Organization (WHO) definition for cholera reporting

- In areas of cholera outbreak : any person age ≥2 years presenting with acute watery diarrhea - ≥3 loose or watery, non-bloody stools within a 24-hour period and severe dehydration or dying from AWD.

- Cholera outbreak declared: any person presenting with or dying from AWD.

Vibrio cholerae

- Highly diverse species with more than 200 serologic groups
- Pathogenic and non-pathogenic variants
- Toxin-producing strains of *V. cholerae* serogroups O1 and O139
- The case-fatality ratio - half in vulnerable groups

Although widely under-reported, the WHO estimates a total of 2.8 million cases with 91,000 deaths annually.

- Key factors- Unique genetic makeup - adapt rapidly to adverse environmental conditions and resist the detrimental effect of antimicrobial agents
- Cholera seasons - high temperatures, heavy rainfall

But cholera can occur year-round

Classification

- Kingdom: Bacteria
- Phylum: Proteobacteria
- Class: Gamma Proteobacteria
- Order: Vibrionales
- Family: Vibrionaceae
- Genus: Vibrio
- Species: V. cholerae

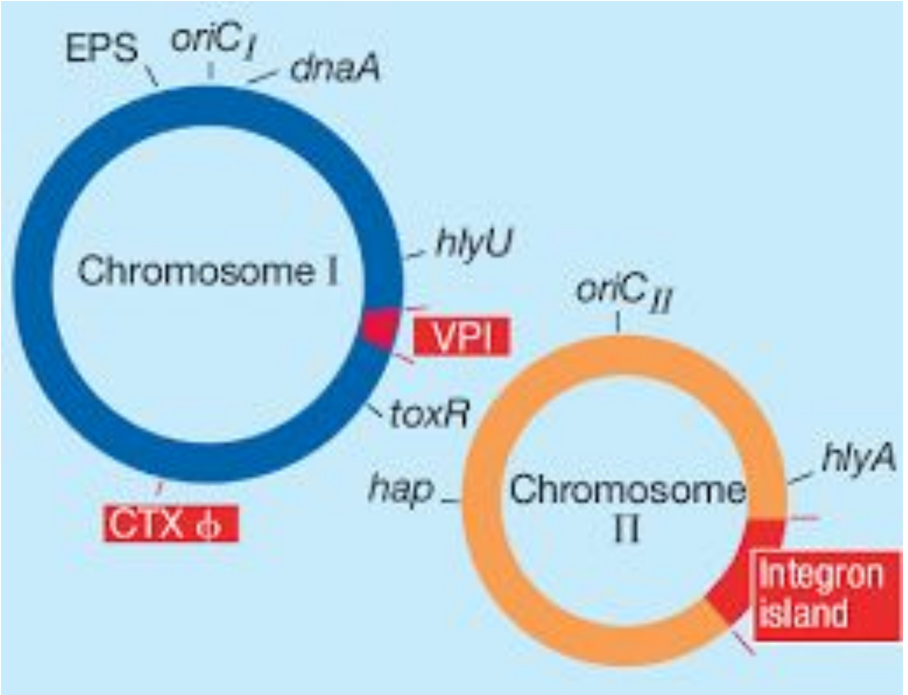
Genome

Two nonhomologous circular chromosomes- Chr1 and Chr2

- Chr 1 -genes for essential cellular functions, virulence genes encoding toxins, adhesins, and surface antigens
- Chr2 -contains a very large integron comprising genes with diverse functions.
- Both- Prophages, genomic islands , and integrative and conjugative elements

Hallmark-ability to exchange genes

Continuous evolution-mobile genetic elements

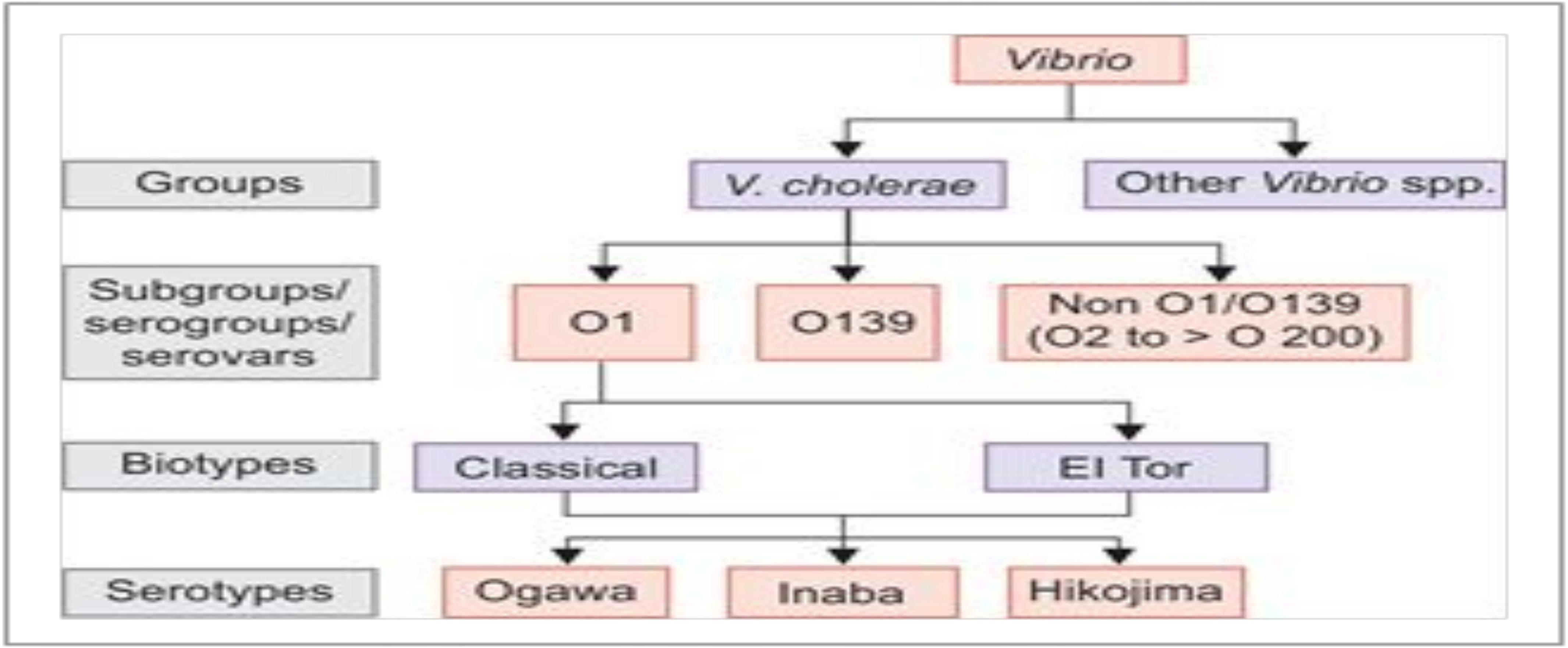


Mobile genetic elements	
Prophage CTXΦ	ctxA and ctxB genes encoding CTX, accessory toxin
Vibrio pathogenicity island-1 (VPI-1)	Toxin coregulated pilus (TCP)
Vibrio pathogenicity island-1 (VPI-2)	Genes for sialidase,transport and catabolism of sialic acid
Vibrio seventh pandemic island-1 (VSP-1	encodes the dinucleotide cyclase (DncV) enzyme, essential for producing intracellular signaling molecule cAMP- GMP ,intestinal colonization
Vibrio seventh pandemic island-1 (VSP-2	type IV pilus
SXT integrative and conjugative element (ICE)	Multiple antibiotic-resistance genes

CLASSIFICATION

Differences in the structure of the O antigen of its lipopolysaccharide

Gardner and Venkatraman Classification:



Characteristics of serotypes

- Ogawa and Inaba co-circulate during epidemics and are capable of interconverting

- Hikojima - rare-unstable transitional form

- Changes may correlate with the immune status of the population-animal models

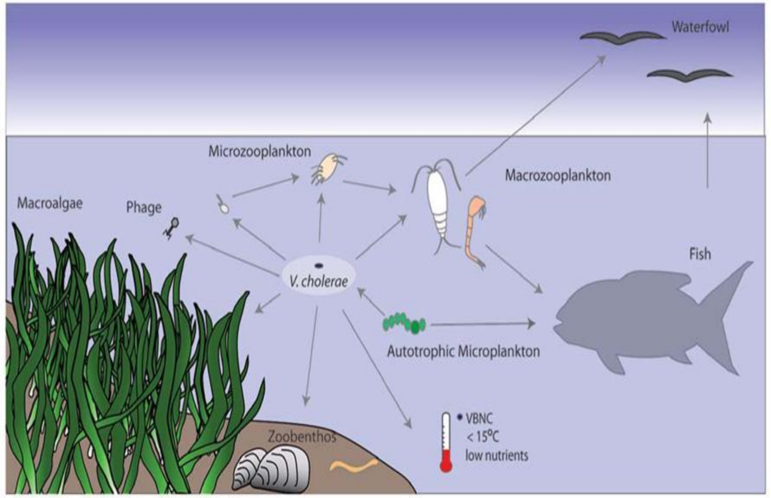
Ogawa serotype

- Affected younger individuals , O blood group , shorter duration of diarrhoea

Inaba serotype

- Blood group A ,had a higher frequency stools.
- More susceptible to tetracycline and erythromycin
- More immunogenic than Ogawa and result in longer-term protection following infection
- The Inaba serotype has been used for the development of live oral cholera vaccine candidates

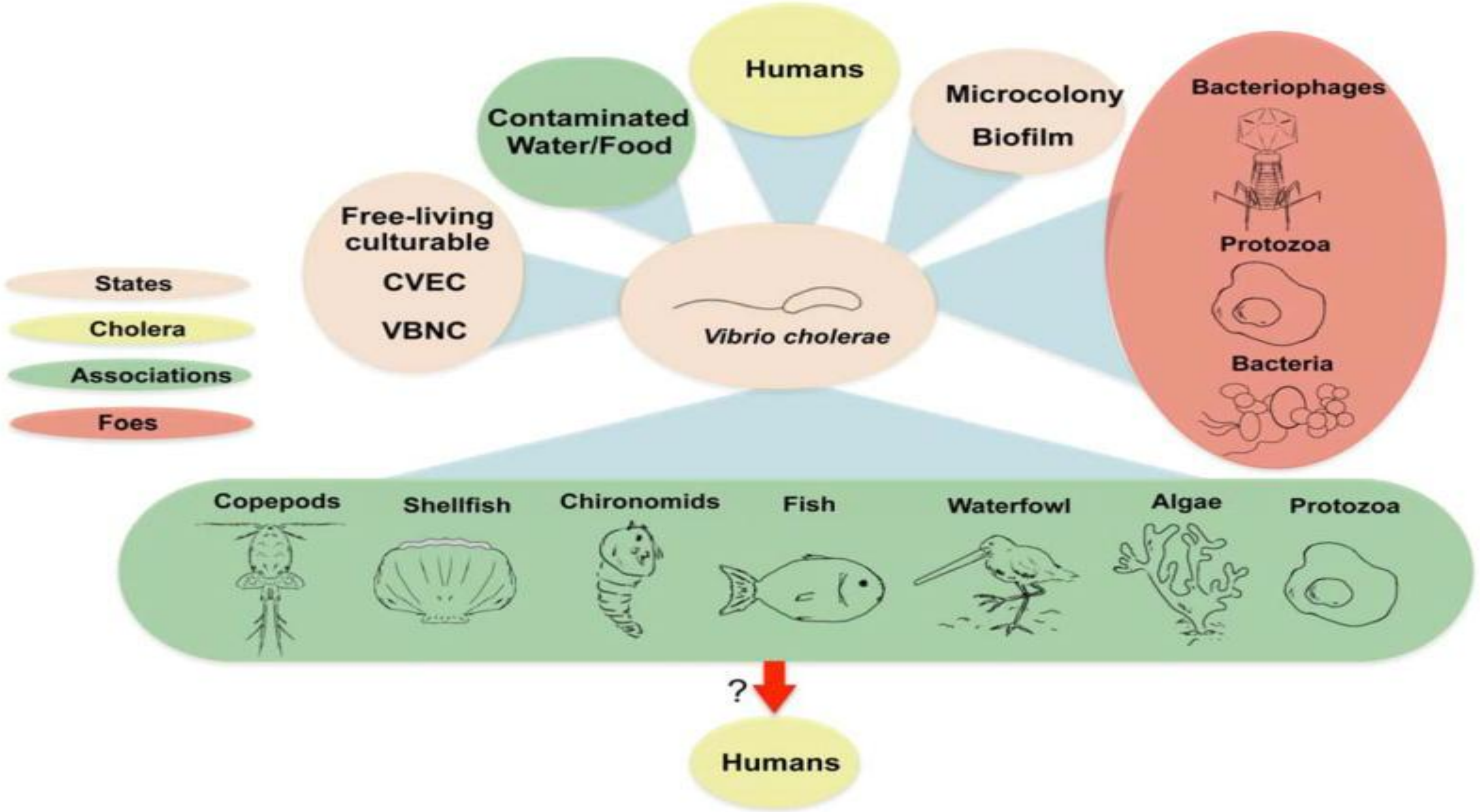
Habitat



Marine environment
brackish riverine, estuarine,
and coastal waters,

Salt loving

close association with copepods
water of lower salinity during warm conditions


States
Cholera
Associations
Foes
Free-living culturable
CVEC
VBNC
Contaminated Water/Food
Humans
Microcolony
Biofilm
Bacteriophages
Protozoa
Bacteria
Vibrio cholerae
Copepods
Shellfish
Chironomids
Fish
Waterfowl
Algae
Protozoa
?
Humans

Two forms

- Viable but not culturable (VBNC) and conditionally viable environmental cells (CVEC)
- VBNC-A dormant state - nutrient deprivation and other environmental conditions

Can cause infection and can revert to the culturable form

- CVEC state in which it can be recovered after the appropriate enrichment

PATHOGENESIS

Transmission

- Outbreaks - contaminated food and water.

- Infectious dose -inoculum of 10^8 *V. cholerae* or higher
- Incubation period -from 12 h to 5 days
- 90–95% -asymptomatic
- 10% develop severe cholera

Toxin mediated

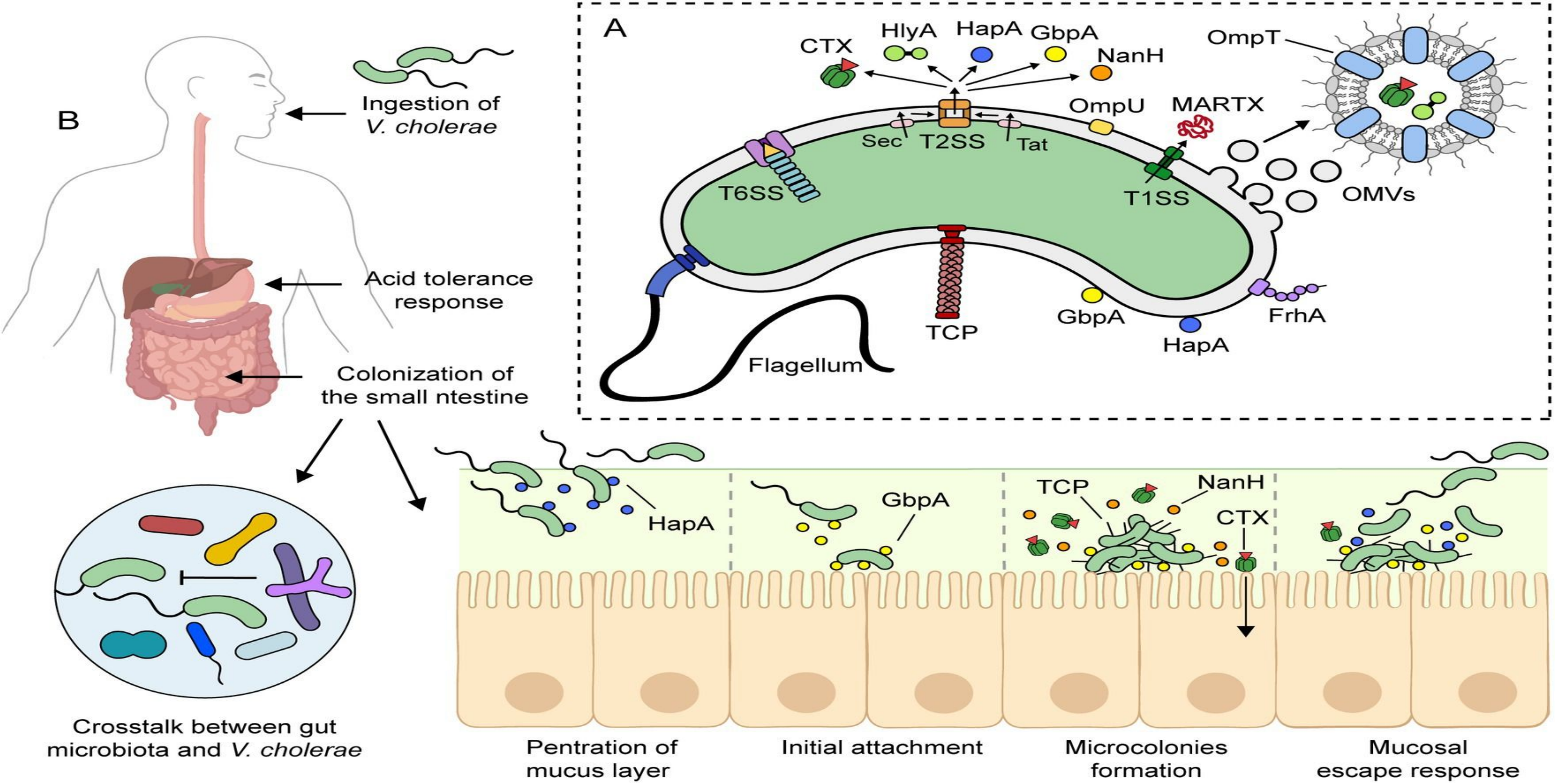
O1 and O139 - cholera toxin

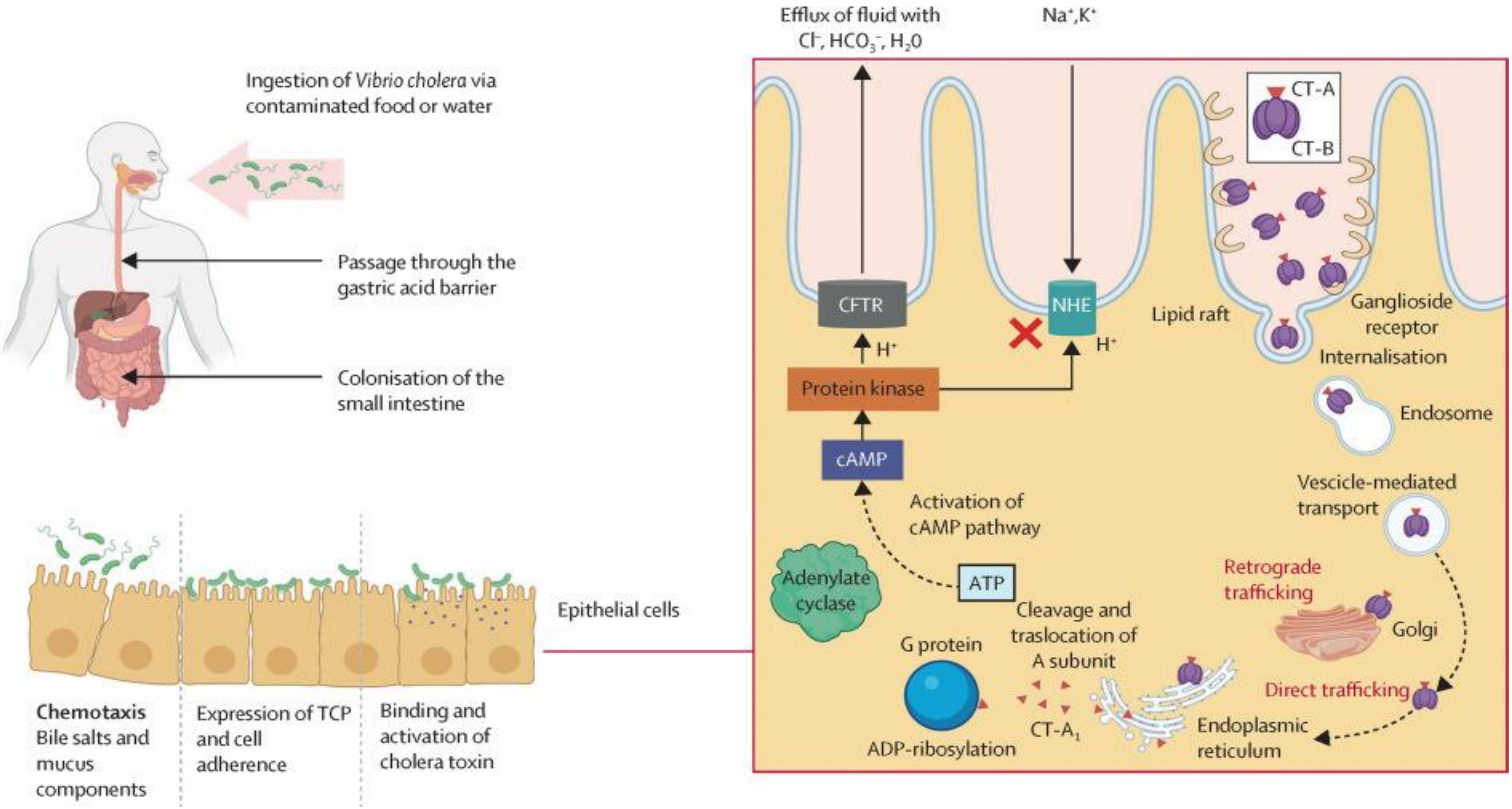
Phage coded a filamentous bacteriophage (CTX) - integrated as prophage into the *V. cholerae* chromosome

TCP -Critical factor mediating colonization

- Bacterium–bacterium interactions - microcolony
- Protection against toxic factors produced in the intestine
- Promote attachment to the intestinal epithelium

Pathogenesis



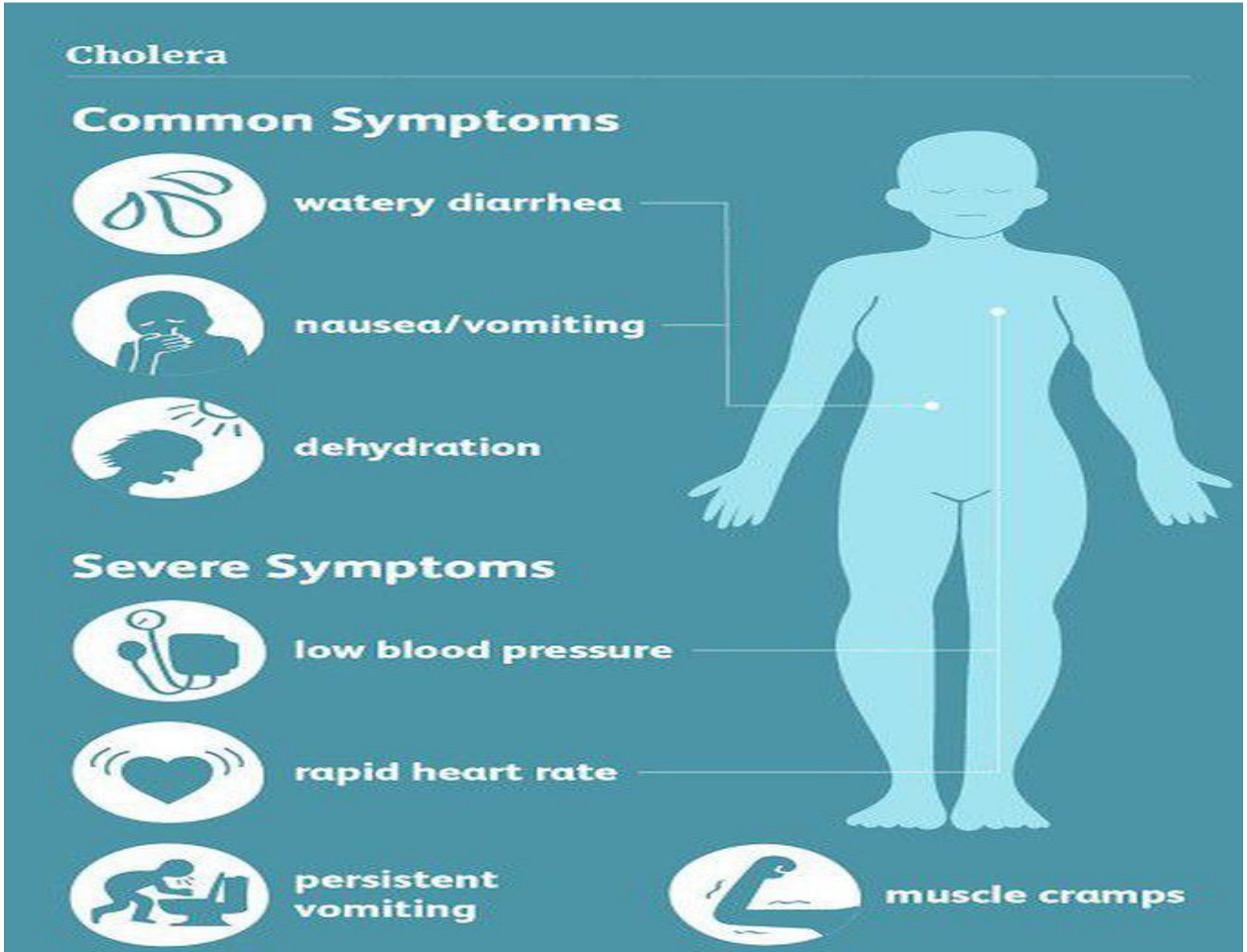


Ingestion of *Vibrio cholera* via contaminated food or water
Passage through the gastric acid barrier
Colonisation of the small intestine
Epithelial cells
Chemotaxis Bile salts and mucus components
Expression of TCP and cell adherence
Binding and activation of cholera toxin
Efflux of fluid with Cl^-, HCO_3^-, H_2O
Na^+,K^+
CT-A
CT-B
CFTR
NHE
H^+
H^+
Lipid raft
Ganglioside receptor
Internalisation
Endosome
Protein kinase
cAMP
Activation of cAMP pathway
Vescicle-mediated transport
Adenylate cyclase
ATP
Retrograde trafficking
Golgi
Cleavage and traslocation of A subunit
G protein
Direct trafficking
CT-A_1
Endoplasmic reticulum
ADP-ribosylation

- **Increase in cyclic AMP -** accumulation of sodium chloride in intestinal lumen Water moves passively into the bowel lumen accumulation of isotonic fluid (watery diarrhea)

- **Loss of fluid and electrolytes** shock and acidosis

Clinical Diagnosis



DEGREE OF DEHYDRATION	CLINICAL FINDINGS
None or mild, but diarrhea	**Thirst in some cases; <5% loss of total body weight**
Moderate	Thirst, postural hypotension, weakness, tachycardia, decreased skin turgor, dry mouth/ tongue, no tears; 5–10% loss of total body weight
Severe	Unconsciousness, lethargy, or “floppiness”; weak or absent pulse; inability to drink; sunken eyes (and, in infants, sunken fontanelles); >10% loss of total body weight

Complications

- Hypovolemia and electrolyte loss
- Pneumonia - aspiration -children.
- Chronic enteropathy and malnutrition - children <5 years
- Pregnancy - increased risk of miscarriage, premature delivery, and stillbirth
- "Cholera sicca" - fluid accumulates in the intestinal lumen; circulatory collapse and death can occur in the absence of diarrhea.
- Bacteremia - rare.

Infections due to Non-O1 /non- O139

- Majority are nonpathogenic-colonise human intestine
- Few produce cholera toxin- Consumption of seafood
- Gastroenteritis may be severe

Never cause epidemic cholera

- Some strains - heat-stable enterotoxin (NAG-ST)

Enterotoxigenic *V.cholerae*

- Encapsulated strains(85%)-Septicaemia
- Wound infections
- Increase in incidence in temperate areas during summer months.

150 YEARS OF CELEBRATING THE MAHATMA

icmr | NICED

INDIAN COUNCIL OF MEDICAL RESEARCH | NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES

आई.सी.एम.आर.– राष्ट्रीय कॉलरा और आंत्र रोग संस्थान

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स्वास्थ्य अनुसंधान विभाग, स्वास्थ्य और परिवार कल्याण मंत्रालय, भारत सरकार

Department of Health Research, Ministry of Health and Family Welfare, Govt. of India

Date : 02.09.2021

To
Dr. Beena Philomina J.
Professor and Head of Department
Dept of Microbiology,
Govt. Medical College,
Kozhikode,
Kerala - 673008

Sub: Identification of the isolates sent to NICED.

Sir/Madam,

Kindly refer to your letter dated 11.08.2021 with aforesaid subject. The two isolates sent by you, has been identified biochemically and serologically. The result is given below.

Sl No	Sample No	Identification of the strain
1.	Not mentioned	Vibrio cholera non O1 non O139
2.	Not mentioned	*Salmonella enterica* serovar Kentucky

Thanking you ,

Yours faithfully,

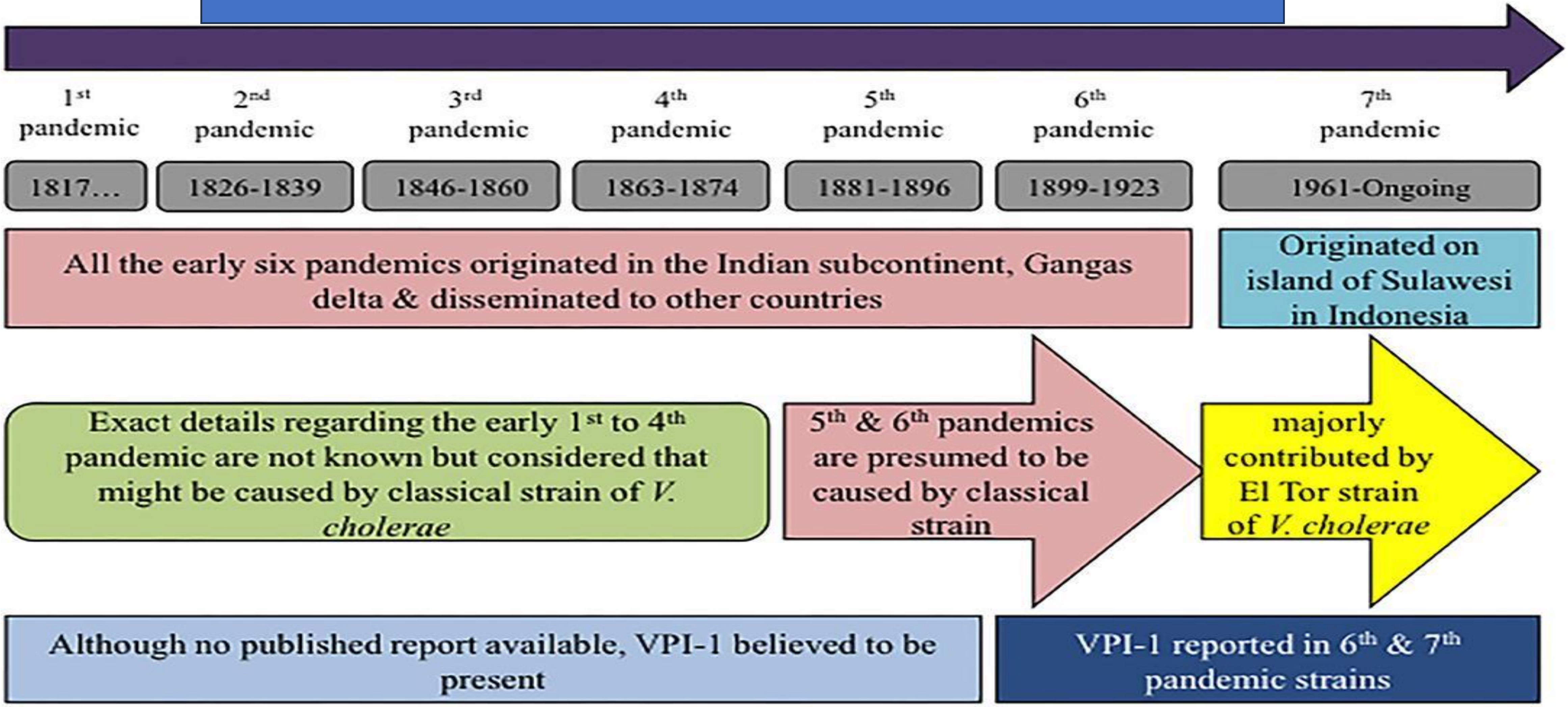
S. Dutta

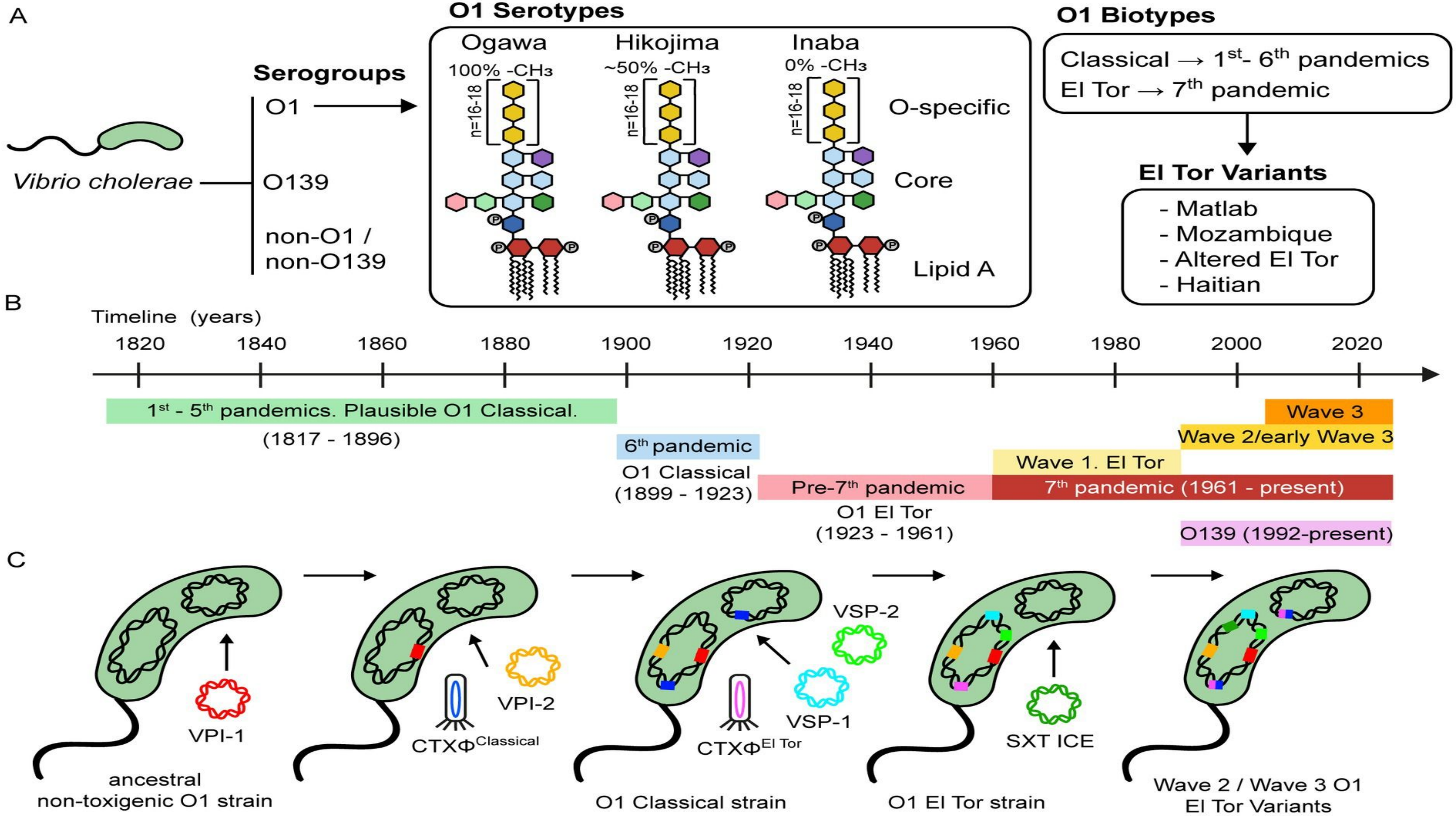
(Dr. S. Dutta, MD, Ph.D)
Scientist G & Director

पी-३३, सी.आई.टी रोड, स्किम-१० एम, बेलियाघाटा, कोलकाता-७०००१०
P-33, C.I.T. Road, Scheme - XM, Beliaghata, Kolkata - 700 010, India
निदेशक / Director : 91-33-2363 3373, 2370 1176, पि बी एक्स / PBX : 91-33-2353 7469/7470, 2370 5533/4478/0448
फेक्स / Fax : 91-33-2363 2398, 2370-5066, वेब / Website : www.niced.org.in

Isolate from a patient with cholera like symptoms

The Pandemics





A
Vibrio cholerae
Serogroups
O1
O139
non-O1 / non-O139
O1 Serotypes
Ogawa
100% -CH3
Hikojima
~50% -CH3
Inaba
0% -CH3
n=16-18
O-specific
Core
Lipid A
O1 Biotypes
Classical → 1st- 6th pandemics
El Tor → 7th pandemic
El Tor Variants
- Matlab
- Mozambique
- Altered El Tor
- Haitian
B
Timeline (years)
1820
1840
1860
1880
1900
1920
1940
1960
1980
2000
2020
1st - 5th pandemics. Plausible O1 Classical.
(1817 - 1896)
6th pandemic
O1 Classical
(1899 - 1923)
Pre-7th pandemic
O1 El Tor
(1923 - 1961)
Wave 3
Wave 2/early Wave 3
Wave 1. El Tor
7th pandemic (1961 - present)
O139 (1992-present)
C
VPI-1
ancestral
non-toxigenic O1 strain
CTXΦClassical
VPI-2
VSP-2
CTXΦEl Tor
VSP-1
O1 Classical strain
SXT ICE
O1 El Tor strain
Wave 2 / Wave 3 O1
El Tor Variants

Seventh Pandemic

- Began in 1961 in Indonesia, and by 1966, affected most of Asia.
- 1971 -observed in Africa and Europe, which had been free of cholera for >100 years

- Three independent waves of transmission

- New genetic material conferring enhanced environmental stability, drug resistance

ElTor and its variants

- The El Tor strains -more adapted and resilient

- More infectious, efficient at host-to-host transmission, survive better in the human gut

- Asymptomatic carriers

- New *V. cholerae* O1 variants carrying mixed classical and El Tor phenotypes
- First isolated from hospitalized patients with severe watery diarrhoea in Matlab, Bangladesh, in 2002

- Belonged to El Tor lineage
- Mozambique in 2004
- Mixed phenotypes of classical and El Tor biotypes - - hybrid biotype or El Tor variant
- polymyxin B susceptibility and positive for chicken erythrocyte agglutination and Voges-Proskauer test
- -polymyxin B -R , positive for CCA and VP test but carrying *ctxB*^{C}

Matlab and Mozambique variants

Emergence of Haitian variant genotype and altered drug susceptibility in *Vibrio cholerae* O1 El Tor-associated cholera outbreaks in Solapur, India-2016

O139 (Bengal Strain)

- October 1992, *Vibrio cholerae* O139 , was detected in Chennai India.

- **Spread along the coastal region of Bay of Bengal**
- Derivative of O1 El Tor – with a **distinct LPS** & **capsulated, toxin**
- The genes encoding O1 antigen biosynthesis replaced by genes encoding the O139 O-antigen

- **Invasive** bacteremia and extraintestinal manifestations

- By 1994 - O1 El Tor replaced O139

Why the potential eighth cholera pandemic *V. cholerae* O139 emerged so successfully and then died out.

Nature Communications Thandavarayan Ranmamurthy et al 2022

Two key genomic evolutionary changes

- A switch from a homogenous toxin genotype in wave-A to heterogeneous genotype
- A gradual loss of antimicrobial resistance (AMR) with the progression of waves.

Role of carriers-Haitian outbreak

- 2010, a severe cholera outbreak began in Haiti

- >800,000 individuals, resulting in thousands of deaths.

- Likely introduced into Haiti by United Nations security forces from Asia
- Asymptomatic carriers of V. cholerae – transmit cholera over long distances.

Situation in India

- In India, cholera is endemic
- Prevalent in the hot, humid and rainy season.
- West Bengal no longer the homeland,all states affected
- Mortality and morbidity has greatly reduced

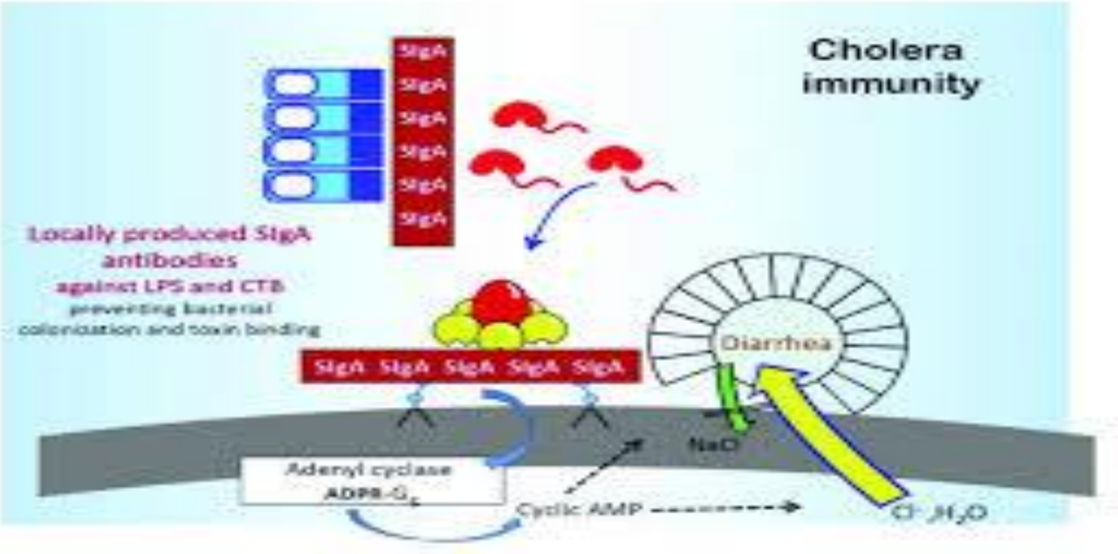
Cholera outbreak comes knocking to Bengaluru amidst acute water shortage-2020 >20 confirmed cases

2023 -5 cholera cases, 1 death in state this year; Mumbai keeps disease at bay

Immunity

- Infection with V.C 01 induces protection against reinfection for at least 3 years
- No protection against V. cholerae O139 and vice versa
- Protective immunity - serogroup specific and toxin independent

The major targets of the human antibody response to *V. cholerae* O1 - O-specific polysaccharide and cholera toxin



Laboratory diagnosis

Darkield microscopy - rapidly evaluation -watery stools

characteristic “shooting star” motility

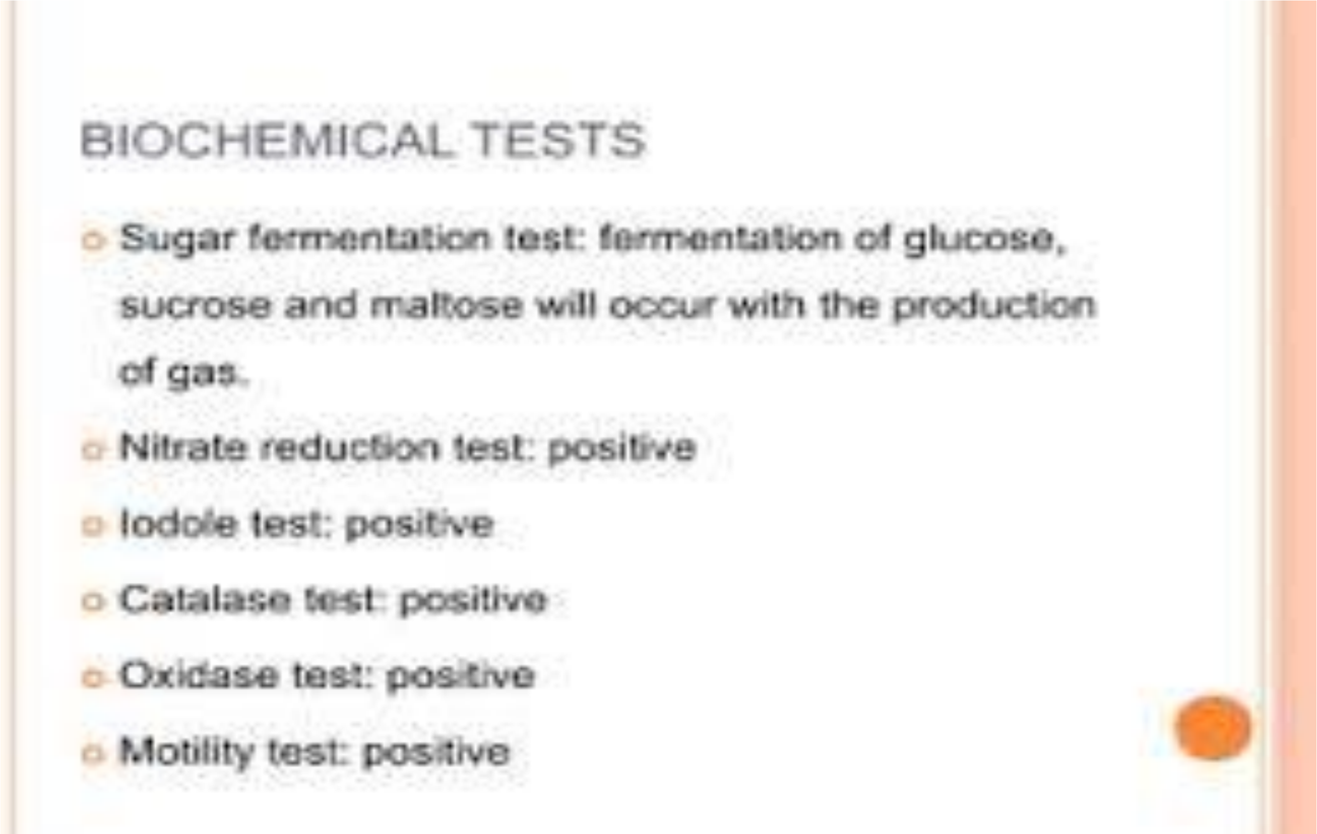
inhibited by the action of specific antibodies

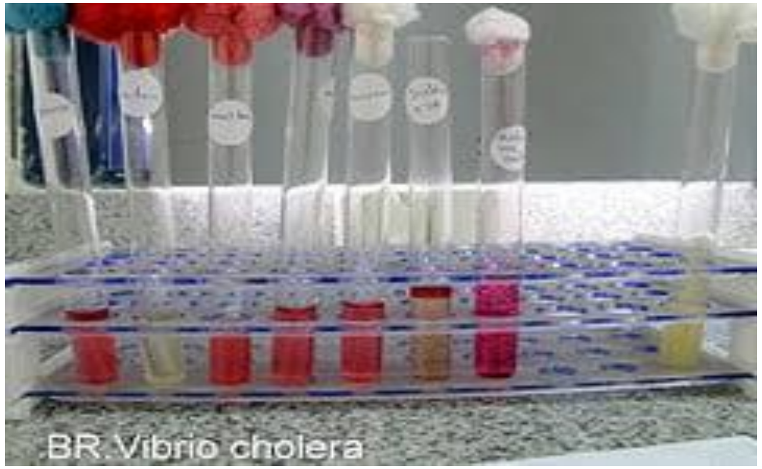
Specimens: Watery stool or rectal swab (for carriers)-Gold standard

- **Transport media:** Cary-Blair medium,Alkaline peptone water
- **Direct microscopy** **Hanging drop –Darting motility**

- **Culture**
  - Enrichment broth: Alkaline peptone water, Monsur’s taurocholate tellurite peptone water
  - Selective media: Bile salt agar, Monsur’s GTTT agar, TCBS agar
  - MacConkey agar

A. *Vibrio cholerae* on blood agar (hemodigestion);
B. **B**. TCBS agar with yellow colored colonies of *Vibrio cholerae;*
C. **C**. String test.





Serogrouping - O1 and O139

L A O

Confirmation –Agglutination with antisera

TABLE 35-2 Differential characteristics of *Vibrio cholerae* biotypes

Properties	*Vibrio cholerae* biotype	
	Classical	Eltor
Hemolysis of sheep RBCs	–	+
Agglutination of chick erythrocytes	–	+
Voges–Proskauer test	–	+
Polymixin B sensitivity	+	–
Susceptibility to		
Mukerjee Group IV Phage	+	–
Eltor phage 5	–	+
Vibriostatic (O/129) agent	+	–

Serotyping: Ogawa, Inaba

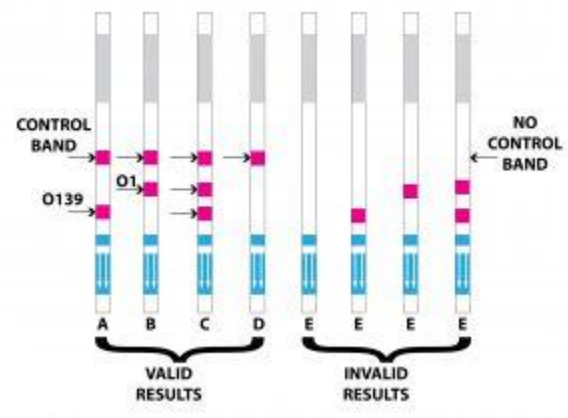
Biochemicals – DD

Acute watery diarrhea	**Enterotoxigenic *Escherichia coli* (ETEC)**	**Most common cause of acute watery diarrhea**
	Vibrio cholerae O1 or O139	
	Norovirus	Vomiting may be a prominent feature
	Campylobacter species	
	Nontyphoidal *Salmonella enterica*	
	Aeromonas species	
	Enteroaggregative *Escherichia coli* (EAEC)	
	Enterotoxigenic *Bacteroides fragilis*	

Antigen detection -POC

- Lateral flow immunochromatographic assay - lipopolysaccharide of V. cholerae O1 and O139

- Antibody-based immunochromatographic dipstick assays (for stool or rectal swab) - O1 or O139 antigen



improved if tested after enrichment in 1% APW for six hours

"Rapid LAMP based Diagnostic Test (RLDT)- point-of-care test

- Directly from the fecal samples - cholera toxin gene and O1 gene could be detected in less than an hour
- Environmental samples
- Applied in resource-poor endemic settings for rapid, sensitive, and reliable diagnosis of cholera
- Biofire Film Array -PCR panel for detection of gastrointestinal pathogens

Antibiotic susceptibility testing

CLSI M45

Agents to Consider for Primary Testing

For *V. cholerae:*
Ampicillin
Azithromycin
Chloramphenicol
Sulfonamides
Tetracycline or doxycycline
Trimethoprim-sulfamethoxazole

Most *V. cholerae* O139 strains and many O1 El Tor strains are resistant to trimethoprim-sulfamethoxazole

Management and Treatment

Eradication of cholera from the world -potentially achievable goal.

Environmental reservoir- complete eradication difficult

WASH

Integrated control programs –Cholera vaccines

Treatment

Primary focus -fluid management

Antibiotics –an adjunct to hydration

- Used in patients with moderate or severe dehydration

a.Reduces the volume of diarrhea by 50% and shortens the duration to 48 hrs

b.Limit the shedding of organisms and may decrease the transmission

- **Tetracyclines** - Doxycycline and tetracycline
- Reduced stool output, duration of diarrhea, vomiting, and requirement for ORS

- **Fluoroquinolones**

- **Macrolides** – Azithromycin and erythromycin

- *V. cholerae* O1 strains with macrolide resistance rare
- Majority *V. cholerae* O139 ,O1 El Tor strains are resistant to trimethoprim sulfamethoxazole and furazolidone

Drug resistance in *Vibrio cholerae*

Acquisition of extrachromosomal mobile genetic elements from closely/distantly related bacterial species

1970-MDR *V. cholerae* O1 R to tetracycline,streptomycin and chloramphenicol was first reported

1977-78 - tetracycline resistant *V. cholerae* O1 cholera epidemic in Tanzania mainly due to the prophylactic use

1994 -death of about 12,000 Rwandan refugees in Eastern Zaire was attributed due to tetracycline resistant *V. cholerae* O1

- 1988–89, Bangladesh – Classical biotype resistant to tetracycline, El sensitive
- 1990 -A drastic increase in resistance against ampicillin , nalidixic acid, chloramphenicol and tetracycline
- 1993-*V. cholerae* O139 from Karachi, Pakistan was reported to be resistant to tetracycline
- 1996-MDR *V. cholerae* O139 R –Sulphamethoxazole.Trimethoprim and Streptomycin -SXT ICE

ICEs played important role in the emergence of multidrug resistant *V. cholerae*

Kerala -Scenario

- Outbreak O1 Ogawa- reported among migrant workers in 2017
- Four people diagnosed with cholera, one succumbed to death
- Virulence genes -toxR and ctxA, were detected in the river and ground water samples collected from the outbreak sites
- MDR -The isolates were susceptible only to tetracycline

- 2019-May 5 patients-GMC,Kozhikode affected with *Vibrio cholerae* El Tor Ogawa

- All were susceptible to tetracycline ,erythromycin and ciprofloxacin and resistant to cotrimoxazole

Cholera vaccines

Cornerstone in WHO's global strategy found in "Ending Cholera: A Global Roadmap to 2030."

- maximize mucosal responses- oral killed vaccines and live attenuated vaccine

CDC

- people traveling to or living in areas of active cholera transmission

WHO -where local transmission of cholera occurs

- During humanitarian crises with a high risk of cholera
- During cholera outbreaks

Shanchol vaccine	Euvichol vaccine/Euvichol Plus vaccine	Dukoral vaccine

mORC-Vax vaccine	OraVacs vaccine

In Vietnam and China

Vaccines under development –
Cholvax vaccine (bivalent, whole-cell, licensed in Bangladesh) and HillChol vaccine (monovalent, whole cell)
Single strain whole-cell of recombinant Hikojima strain-MS1568

CVD 103-HgR-Live attenuated

CholeraGarde vaccine	Vax-COLER vaccine

Live

Oral Cholera Vaccines:

Killed whole-cell vaccine:Shanchol-Licensed in 2009 in India

- **biotypes and serotypes of V. cholerae O1 and O139 without cholera toxin B subunit**
- Two doses are given orally, >1 year age
- **Protection:** For 3 years.

- **Recombinant B subunit vaccine** –WC-rBS Dukoral
- Both biotypes and serotypes of V. cholerae O1 supplemented with 1 mg of recombinant cholera toxin B subunit per dose. Licensed in 1991 in Sweden
- Two doses are given orally, with minimum of one week gap. A third dose is given for children aged 2-5 years.
- **Protection:** 2 years

Euvichol /Euvichol plus-Korea

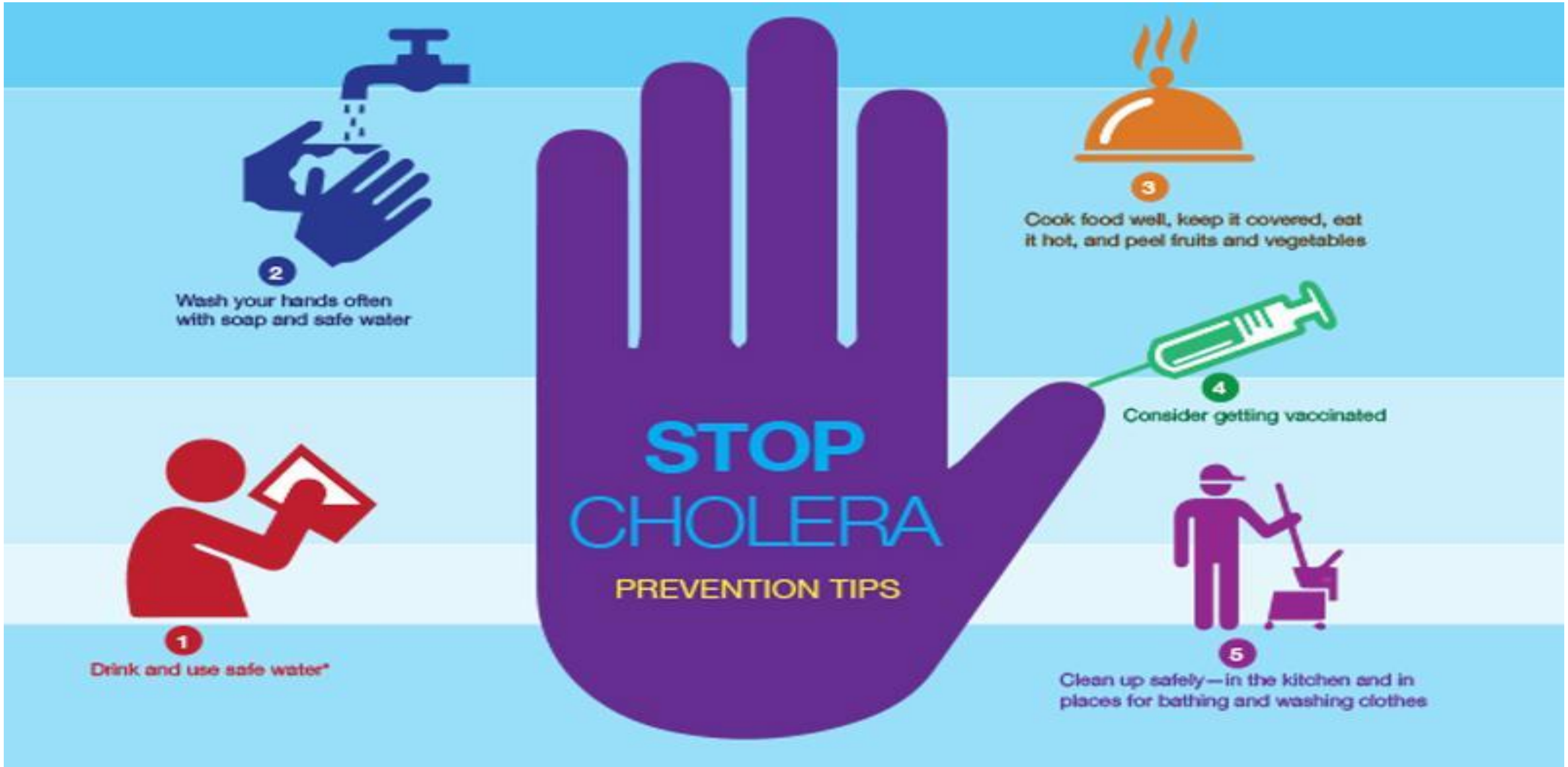
Oral live attenuated vaccines –Lack the genes for cholera toxin

- **CVD 103-HgR** : given as single oral dose-10 days before travel
- age 2-64 years, travellers

90% protection at 10 days after vaccination; which lasts for 3-6 months.

Cholera is a preventable and treatable disease

- In 2017, the Global Task Force for Cholera Control -to eliminate endemic cholera in 20 countries and reduce cholera deaths by 90% by 2030
- “Ending Cholera: A Global Roadmap to 2030,” –
- strengthening public health systems
- improving surveillance for early detection of cholera outbreaks,
- improving drinking water, sanitation, and hygiene conditions,
- making oral rehydration treatments more accessible, and increasing vaccination coverage.


STOP
CHOLERA
PREVENTION TIPS
1
Drink and use safe water*
2
Wash your hands often
with soap and safe water
3
Cook food well, keep it covered, eat
it hot, and peel fruits and vegetables
4
Consider getting vaccinated
5
Clean up safely—in the kitchen and in
places for bathing and washing clothes

THANK YOU