

**Le rôle des *Vibrio* spp.  
chez l'homme**

**De rol van *Vibrio* spp.  
bij de mens**

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Belgian NRC Vibrio**

# *Vibrio* spp and men

- **Introduction**
- **Clinical issues and bacteria**
- **Epidemiology of vibriosis**
- **Diagnosis in the clinical biology lab**
- **Confirmation by the National Reference Centre (*R.Sacheli*)**



# INTRODUCTION

# *Vibrio* spp and Men

Human infections with pathogenic species of the family *Vibrionaceae*

- Illnesses caused by *Vibrio cholerae* O1 and O139 strains that produce cholera toxin are defined by the World Health Organization (WHO) as **CHOLERA**.
- Illnesses caused by other *Vibrio* strains are called **VIBRIOSIS**.



# ***Vibrio* spp and Men**

## **Human infections with pathogenic species of the family *Vibrionaceae***

### **■ Cholera**

- **Acute diarrheal illness caused by infection of the intestine with a toxigenic strain of *Vibrio cholerae* O1 or O139**
- **Estimated burden : 1.4 to 4.3 million cases each year**
- **Over 100,000 death per year worldwide**

### **■ Vibriosis**

- **Infection with serogroups of *V. cholerae* non-O1, non-O139 or with other species from the family *Vibrionaceae***
  - *V.parahaemolyticus, V.alginolyticus, V.vulnificus, V.fluvialis, etc.*
- **Typically foodborne disease by eating contaminated raw or undercooked shellfish / seafood products**
- **Non-bloody diarrhea, bloodstream infections, wound infections, necrotizing fasciitis**

- **Security consideration for**

- **Handler**
- **Cook**
- **Consumer**



- **Biosecurity precautions for**
  - **Handler of raw products to control**
  - **Lab technician**

**Cholera & *Vibrio cholerae***  
**Vibriosis & *Vibrio* spp**

# **CLINICAL ISSUES & BACTERIA**



# Cholera



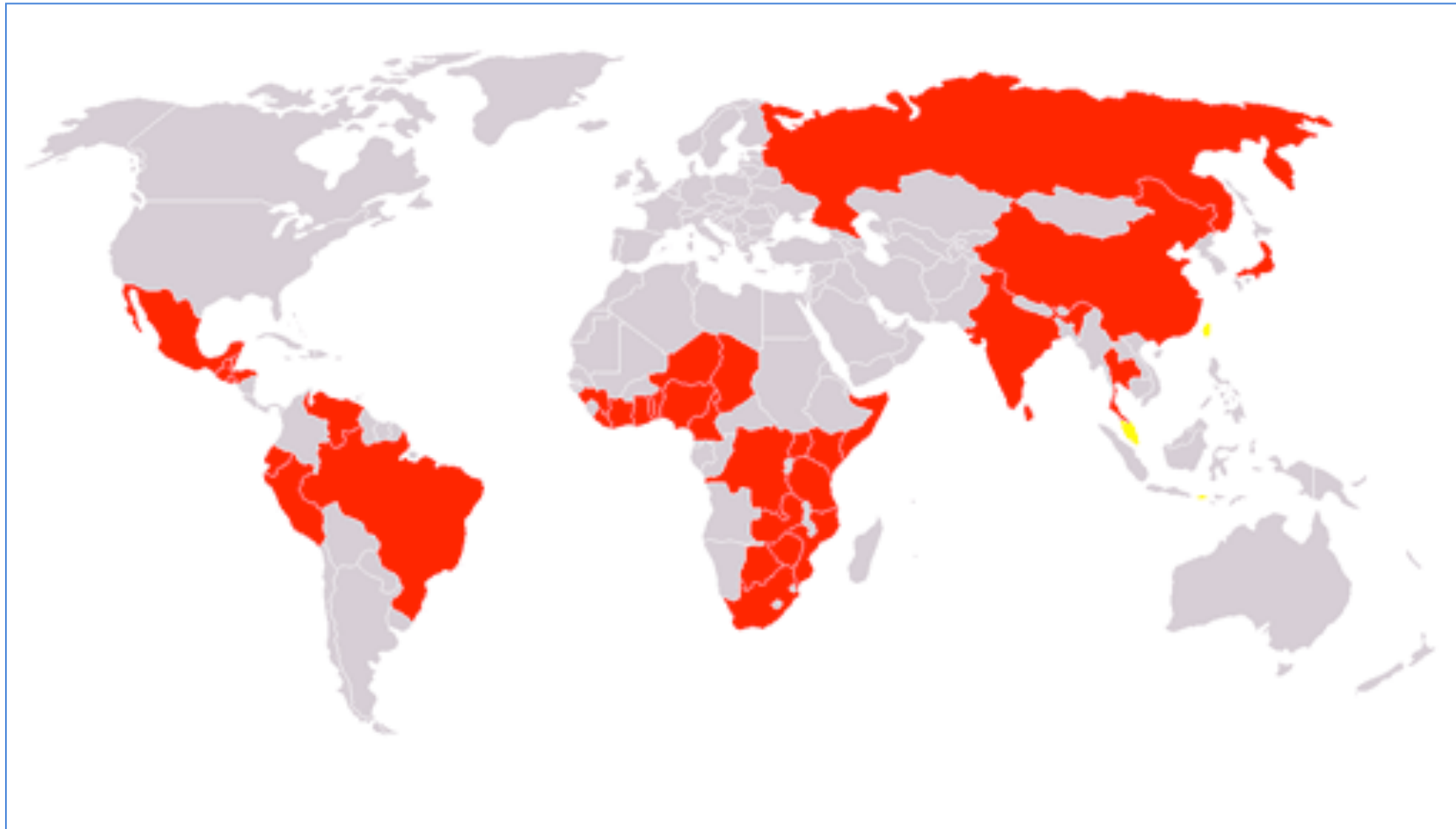
# Cholera

**Acute diarrheal illness caused by infection of the intestine with a **toxigenic strain of *Vibrio cholerae* O1 or O139****

**Following ingestion of contaminated food or water**

- **Long history of epidemics and pandemics**
  - **During the 19th century, Cholera spread across the world from its original reservoir in the Ganges delta in India.**
  - **Six subsequent pandemics killed millions of people across all continents**
  - **The current (seventh) pandemic started in South Asia in 1961, and reached Africa in 1971 and the Americas in 1991**
- **Now endemic in many countries, in SE Asia, Africa, South and Central America**

# Cholera



# Cholera

Acute diarrheal illness caused by infection of the intestine with a **toxigenic strain of *Vibrio cholerae* O1 or O139**

- **Estimated burden each year**
  - 3-5 millions cases; 20,000 to 140,000 deaths worldwide (*WHO 2015*)
- **Remains a global threat to public health**
- **Mainly in population where**
  - Lack of safe water
  - Lack of sanitation facilities and sewerage system
  - Precarious hygiene







# Cholera & *Vibrio cholerae*

## ■ Pathogenecity

- Acute watery non-bloody diarrhea with severe dehydration, sudden onset +/- vomiting and abdominal cramps
- Very serious disease “*Cholera gravis*” & mild disease
  - Afecal stool like « eau de riz »
  - 10 - 50 stools /d
  - Up to 1L /hour; 3-15 L /d !!
  - Loss of electrolytes resulting in
    - Severe dehydration
    - Metabolic acidosis (loss of bicarbonates)
    - Hypokaliemia (loss of K)
    - Hypovolemic shock
    - Cardiac failure
- Asymptomatic or mild affected people
  - *Vibrio* in their stool for 1-10 days → shed back in the environment !





# Cholera

- **Mortality**
  - **Up to 60% if dehydration not promptly treated**
    - **Affects both children and adults**
    - **Vulnerability of children < 5 y and oldest people**
  - **< 1% if dehydration rapidly treated**
  
- **Incubation**
  - **Between 12 hours and 5 days**
  - **Illness : 2-7 days**



# *Vibrio cholerae* (Vibrionaceae)

Biosafety Level 2 (BSL-2) pathogen

## Characteristics

- Gram negative bacilli, curved
- 0,5 - 0,8  $\mu\text{m}$  to 3  $\mu\text{m}$
- A unique polar flagellum → high mobility
- Facultative anaerobe
- Oxydase +

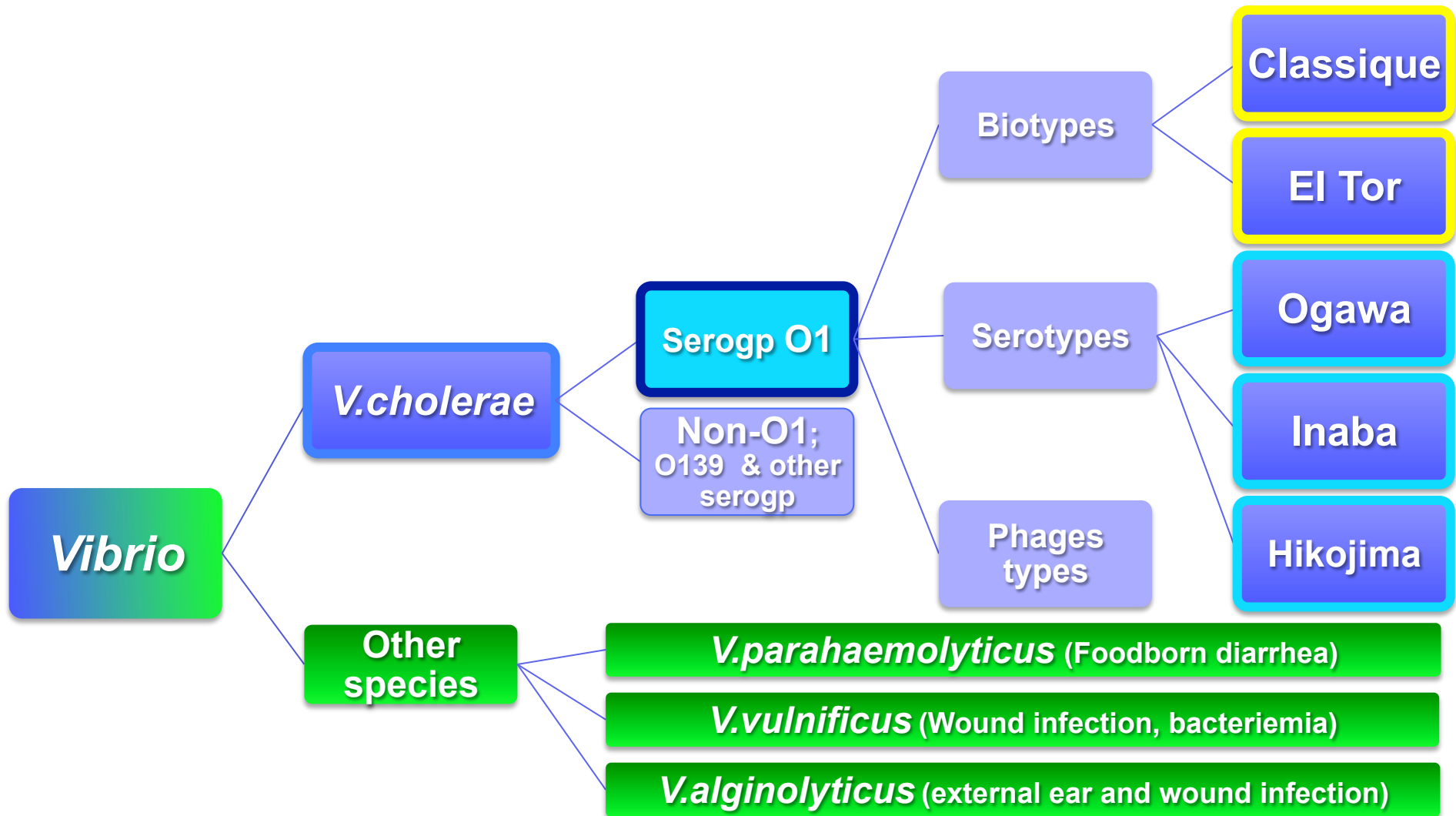
## Many serogroups (>200)

- Only two, O1 and O139 → outbreaks
  - No difference in illness
- O139, never found out of Asia





# *Vibrio cholerae*: O antigen-based classification

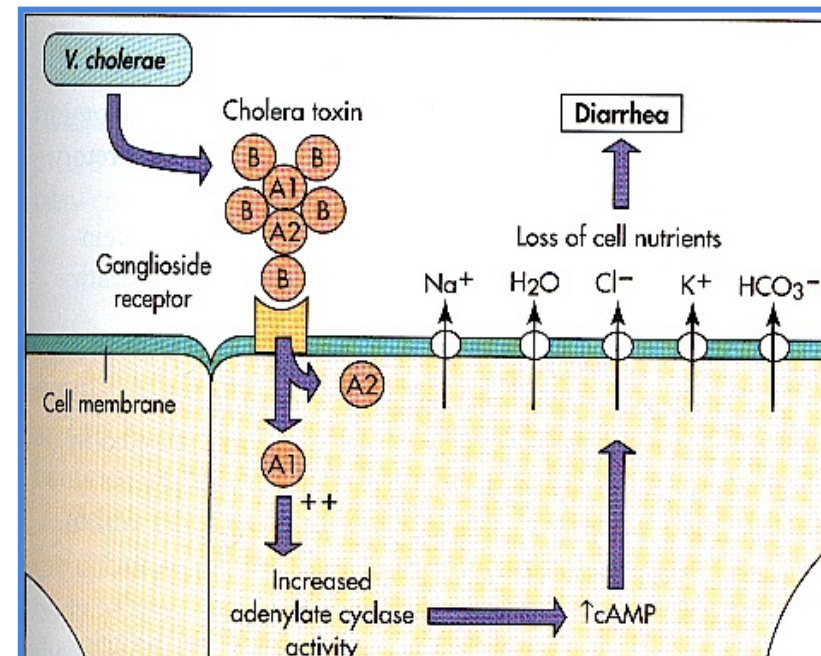




# Vibrio cholerae

- **Virulence factors**
  - **Mobility (flagellum)**
  - **Adhesion to intestinal epithelial cells of the small bowel**
    - Successive expression of several pathogenic factors
      - Production of mucinase
      - Attachment to specific receptors
  - **Production of the potent polymeric enterotoxine**
    - **Watery diarrhea**
    - **Production linked to the integration of the CTX bacteriophage**

**Hyperactivation → Massive loss of electrolytes**





# *Vibrio cholerae*

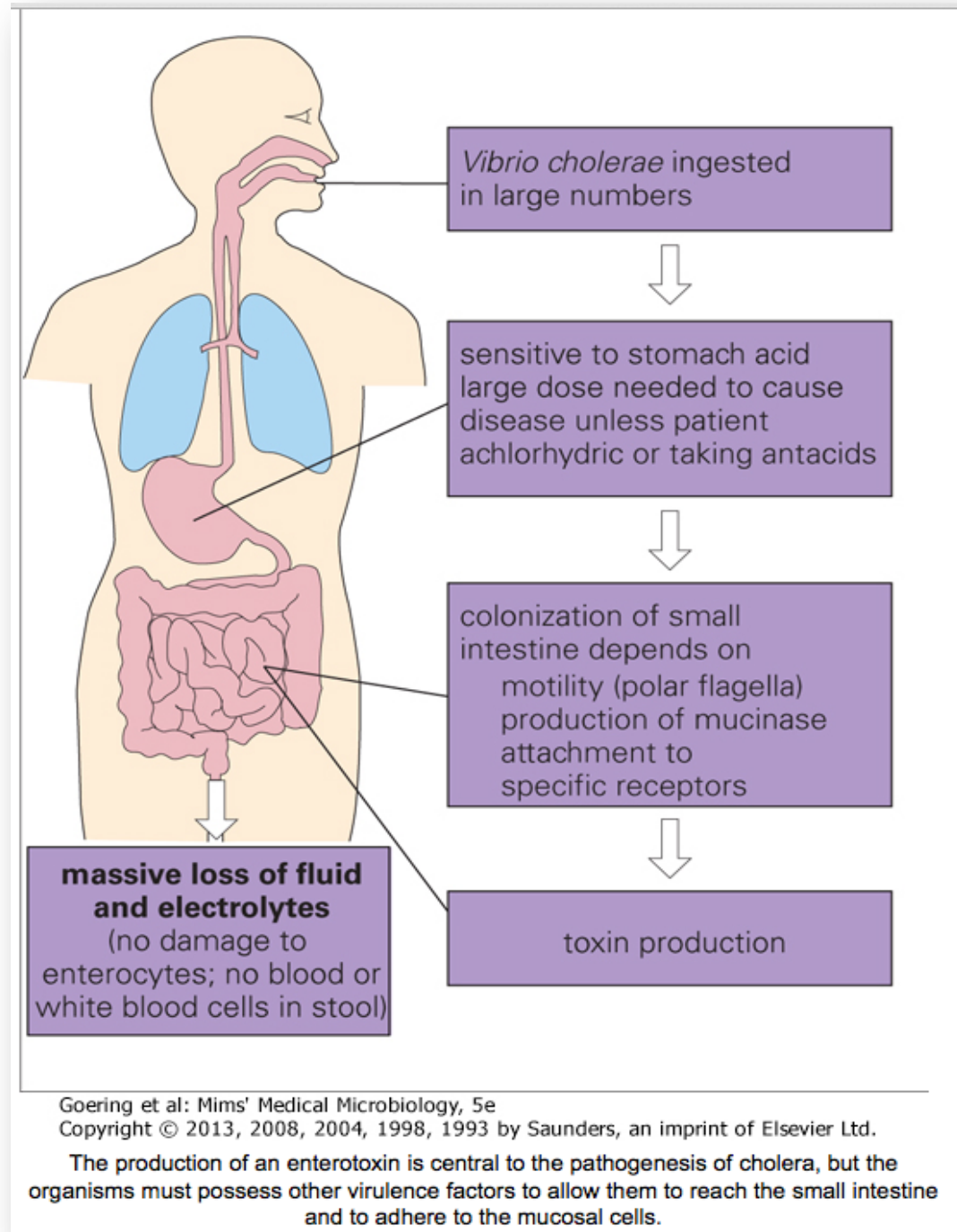
- **Virulence factors**
  - **Mobility (flagellum)**
  - **Adhesion to intestinal epithelial cells (small bowel)**
    - Successive expression of several pathogenic factors
      - Production of mucinase
      - Attachment to specific receptors
  - **Production of the enterotoxine**
    - **Watery diarrhea**
      - Up to  $10^6$ - $10^8$  vibrio /ml of stool
    - Production linked to the integration of the CTX bacteriophage
- **Infecting dose**
  - **Around  $10^8$  ( $10^6$ - $10^{11}$ )**
    - Less if ↓ of gastric acidity (positive experiment with  $10^4$ )
    - People with blood group O more susceptible



## Toxigenic *V.cholerae*



**Massive loss of  
liquid and of  
electrolytes  
(no leucocytes neither  
blood in stool)**







# *Vibrio cholerae*

## ■ Reservoirs

- People and aquatic sources somewhat salted such as estuaries and some coastal areas
- Stagnant water, contaminated by sewage
- Infection only in man
- Important reservoir: asymptomatic infected people

## ■ Transmission

- Feco-oral route
- Contaminated food, shellfish farmed in estuaries, ...
- Closely linked to inadequate access to clean water and sanitation facilities
- Typical risk areas: peri-urban slums, camps of refugees, etc.
- Unusual transmission from man to man





# *Vibrio cholerae*

- Reservoirs and transmission





# Control and prevention of Cholera



# Cholera

- **Treatment**
  - **Easy and effective**
    - **Rapid access to treatment is essential**
    - **Oral or intravenous rehydration salts in solutions**
      - **Several litres needed each day**
        - **Per os or I.V. for the more severe cases**
    - **+/- antibiotics (doxycycline, azithromycin)**
      - **To reduce length of illness**
        - **reduction of time of excretion**
        - **reduction of volume of rehydration solution**







## Goal of treatment is REHYDRATION:

- Oral Rehydration Solution (ORS)
- Intravenous Fluids :
  - Lactated Ringers
  - Normal Saline

Oral or IV antibiotics may be necessary in the cases of severe cholera.



# Cholera

## ■ Prevention

### ■ Multifaceted approach

- Provision of safe water
- Daily good hygiene practices
- Public sanitation and sewerage system
- Oral cholera vaccines in conjunction to previous conventional control measures, not replace
- Social mobilisation
- Surveillance





# Poster éducatif, en créole haïtien, pour lutter contre la diffusion du choléra



## Pwoteje tèt nou pou n pa trape kolera



Oktòb 2010

Reproduction autorisée par le Ministère de la Santé Publique et de la Population

# Vibriosis

Typically foodborne disease by eating contaminated seafood,  
or other disease following entry via wound exposure to contaminated seawater

## ■ Etiologic agents

- About a dozen *Vibrio* species can cause human illness
  - *V.cholerae non 01, non 0139, V.parahaemolyticus, V.alginolyticus, V.vulnificus, V.fluvialis, etc.*
- Gram-negative bacilli growing well in salty environments, such as seawater.
- Naturally and commonly found in warm marine and estuarine environments



# Vibriosis

- **Clinical manifestations**
  - Typically characterized by watery diarrhea, usually with abdominal cramping, nausea, vomiting, and fever
  - Also wound or soft tissue infections
- **Risk group**
  - Anyone can get vibriosis
  - At higher risk of infection and serious complications, people
    - With underlying conditions, such as alcoholism and liver disease, cancer, diabetes,
    - Receiving immune-suppressing therapy
    - Taking medicine to decrease stomach acid levels
    - Having recent stomach surgery

# Vibriosis

## ■ Diagnosis

### ■ Suspicion of vibriosis

- if a patient has watery diarrhea and has recently eaten raw or undercooked seafood, especially oysters,
- or when a wound infection occurs after exposure to seawater.

- Infection is diagnosed when **Vibrio** bacteria are found in the stool, wound, or blood of a patient who has symptoms of vibriosis.

## ■ Treatment

- Not necessary in mild cases, but patients should drink plenty of liquids to replace fluids lost through diarrhea.
- Although no evidence that antibiotics decrease the severity or duration of illness, they are sometimes used in severe or prolonged illnesses.

# Vibriosis

## ■ **Transmission**

- **From eating raw or undercooked shellfish, particularly oysters**
- **Wound infections may occur when wounds or soft tissues are exposed to brackish or salt water.**

# Vibriosis

- ***V. parahaemolyticus***
  - Important cause of enteritis
  - Associated with ingestion of raw or improperly prepared seafood

# Florida beachgoers warned about deadly flesh-eating bacteria

## FLESH EATING BACTERIA

- Activated by warm water and salt water
- Exposure to water with open cuts and eating raw shellfish increases risks of infection
- Infection could lead to skin breakdown, amputation and potential death



## Gulf Coast Oysters: A Raw Deal (The Villainy of *Vibrio vulnificus*)



County of San Diego  
Department of Environmental  
Health

# A « flesh-eating bacteria »

*V.vulnificus*



# Vibriosis

- ***V.vulnificus***
  - Typically bloodstream infections in persons with liver disease, cancer or other immune-compromising condition
  - Life-threatening illness
    - half of bloodstream infections rapidly fatal
  - Also wound or soft tissue infections → necrotizing fasciitis : one of the « Flesh eating bacteria »
  - Entry via wound exposure to contaminated seawater or seaproducts



# Vibrio vulnificus infection

including Necrotising Fasciitis  
caused by *V. vulnificus*



contaminated with the bacteria.

## ● Clinical features

Wound infection with *V. vulnificus* may result in necrotising fasciitis (commonly known as 'flesh-eating disease'), which is a serious bacterial infection of the soft tissue and fascia (a sheath of tissue covering the muscle). It can lead to tissue destruction and can be fatal. The mortality rate of persons affected by *V. vulnificus* associated necrotising fasciitis is about 30% locally. Most cases of infection were reported during summer months.

Clinical features of necrotising fasciitis may include intense pain, redness, swelling and rapidly developing tissue destruction. The skin changes can start at the site of injury as trivial as a small cut or bruise, while in other cases there is no obvious source of infection. The level of pain is out of proportion to the visible skin changes.

Consuming food that is contaminated with *V. vulnificus* may occasionally cause diarrhea, vomiting and abdominal pain. In persons with underlying medical conditions, especially liver disease, *V. vulnificus* can infect the bloodstream typically causing fever, chills, decreased blood pressure and blistering skin lesions.

## ● Mode of transmission

*V. vulnificus* infection is acquired from exposure of wounds or soft tissues to the germ that is present in seawater or seafood, or through eating raw or undercooked shellfish, particularly oysters harvested from warm water. No human to human transmission has been reported.

## ● High risk groups

All persons can be affected. People who come in direct contact or handle raw seafood have a higher risk of necrotising fasciitis. Persons with underlying medical conditions, such as hypertension, diabetes mellitus, heart diseases, chronic liver diseases, or those with impaired immune response may also be at increased risk of serious complications.

Appropriate antibiotics given promptly are needed to kill the germ. Some patients may require intensive care. For necrotising fasciitis, in order to stop infection from spreading, surgery e.g. removal of dead tissue or amputation of the limb may be required.

## ● Prevention

Some tips for preventing *V. vulnificus* infection, particularly among people with impaired immune response or with underlying medical illnesses include:

- Avoid having the wound coming in contact with seawater or raw seafood.
- Cleanse the wound thoroughly and cover it with waterproof dressing.
- Avoid skin contact with dirty water when visiting a wet market.
- Be careful with sharp parts of seafood, such as fish fins, shrimp heads and crabs to prevent cuts.
- Use appropriate protective devices (e.g. gloves) when handling raw seafood.
- Avoid eating raw oysters or shellfish.
- Cook seafood thoroughly; for shellfish (e.g. oysters, clams, mussels), cook until the shells open.
- Avoid mixing ready-to-eat food and raw seafood.

If symptoms such as skin redness, swelling and pain develop, seek medical advice promptly.

Centre for Health Protection Website

[www.chp.gov.hk](http://www.chp.gov.hk)

24-hour Health Education Hotline  
of the Department of Health  
**2833 0111**

Produced in October 2013



# **EPIDEMIOLOGY OF VIBRIOSIS**

# Vibriosis

## ■ Incidence in the USA

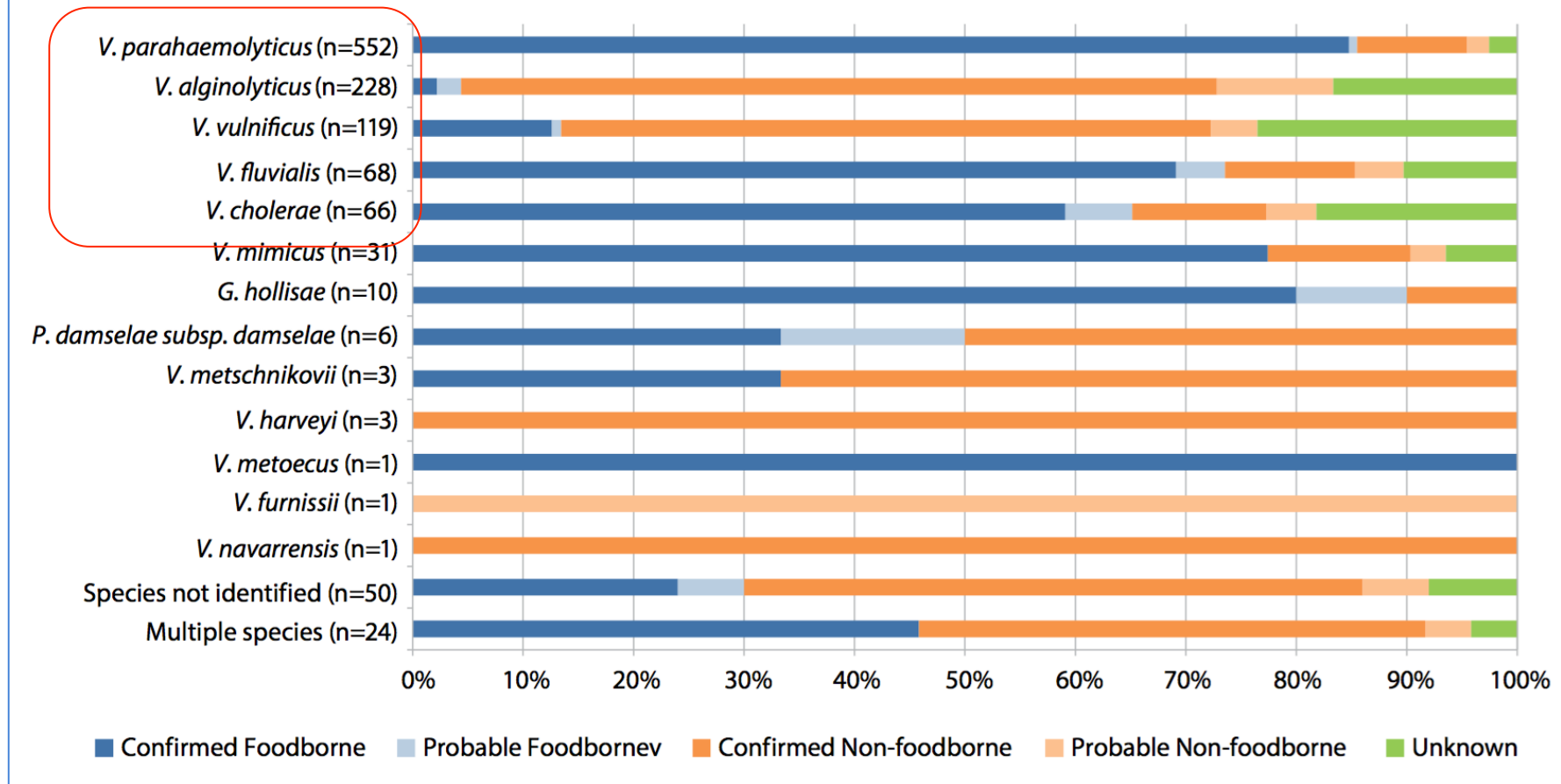
- An estimated 80,000 cases of vibriosis, 500 hospitalizations, and 100 deaths in the United States each year

## ■ Sequelae

- Most people with mild illness typically recover after about 3 days and suffer no long-term consequences.
- *V. vulnificus* can cause particularly severe and life-threatening infections
  - These infections are fatal approximately 50% of the time.
  - Amputation of limbs may be needed for severe wound infections.

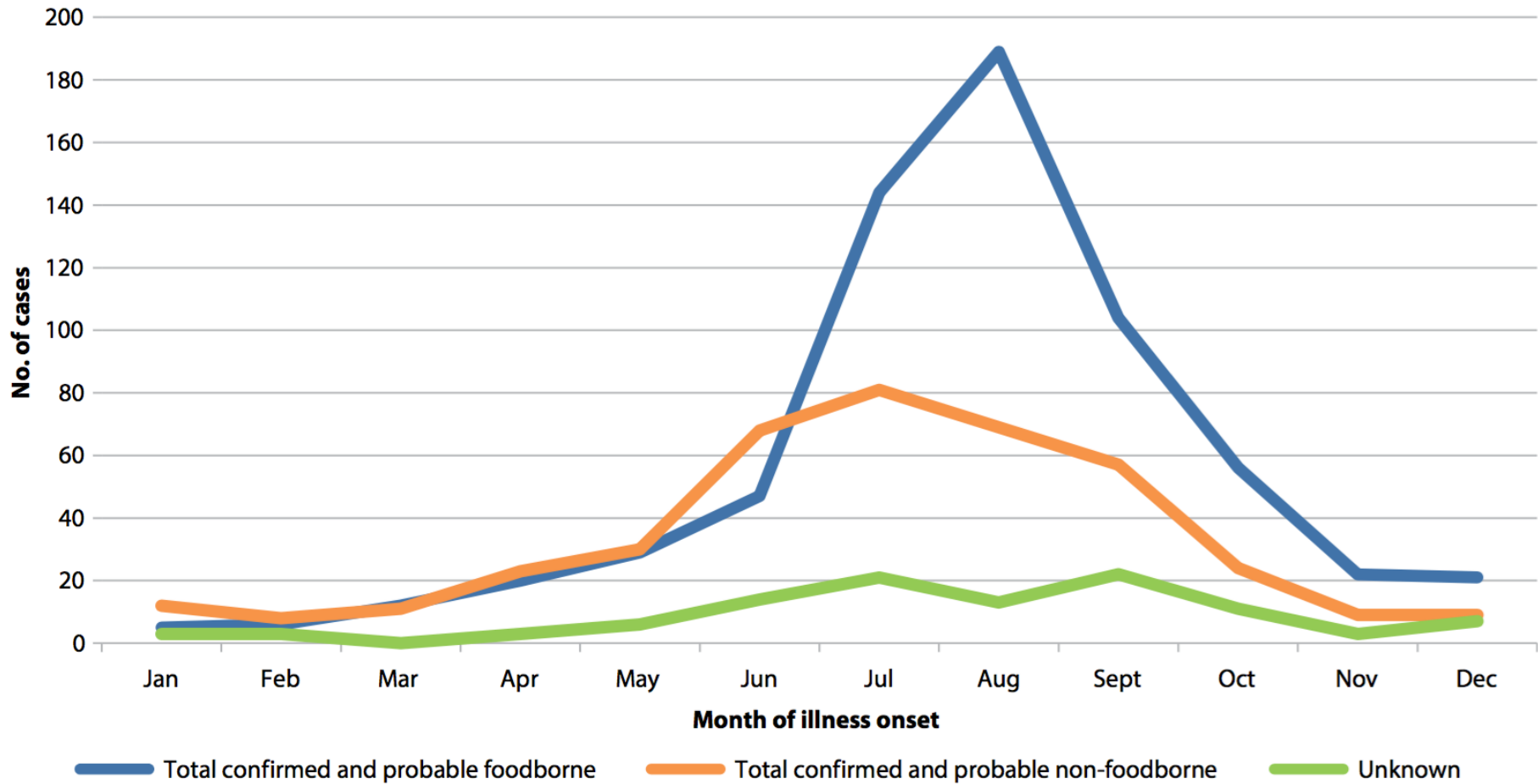
# Transmission and reported exposures

**Figure 2. Domestically acquired vibriosis cases by transmission route and species, United States, 2014 (N=1,163).**



From Cholera and Other Vibrio Illness Surveillance (COVIS) system (a USA national surveillance system for human infection with pathogenic species of the family *Vibrionaceae*, which cause vibriosis and cholera), Report May 2016

**Figure 3. Domestically acquired vibriosis cases, by month of illness onset or specimen collection (when onset date not available), and transmission route, United States, 2014 (N=1,162\*).**



From COVIS, Report May 2016

**Table 2. Seafood exposures among 286 patients with domestically acquired foodborne vibriosis\* who reported eating a single seafood item in the week before illness onset, United States, 2014.**

|  | Mollusks |         |         | Crustaceans |         |        |          | Other             |          |
|--|----------|---------|---------|-------------|---------|--------|----------|-------------------|----------|
|  | Oysters  | Clams   | Mussels | Shrimp      | Lobster | Crab   | Crayfish | Other Shellfish** | Finfish† |
| <b>Patients who ate single seafood item, n (% of 286)</b>                    | 196 (69) | 12 (4)  | 3 (1)   | 16 (6)      | 3 (1)   | 16 (6) | 5 (2)    | 7 (2)             | 29 (10)  |
| <b>Patients who ate the single seafood item raw, n (% of n in row above)</b> | 174 (89) | 10 (83) | 0 (0)   | 2 (13)      | 0 (0)   | 1 (6)  | 0 (0)    | 2 (29)            | 5 (17)   |

\*Includes confirmed and probable foodborne cases.

\*\*Other shellfish reported: conch, scallops.

†Finfish reported: bass, bluegill, catfish, cod, flounder, herring, poke, salmon, squid, tilapia, tuna, walrus, and white fish.

From COVIS, Report May 2016

# DIAGNOSIS IN THE CLINICAL LABORATORY





# *Vibrio cholerae*

## ■ Diagnostic

### ■ Stool specimen

- Afecal stool « eau de riz »
- Under microscope
  - No leucocytes
  - Monomorphic flora : curved bacilli

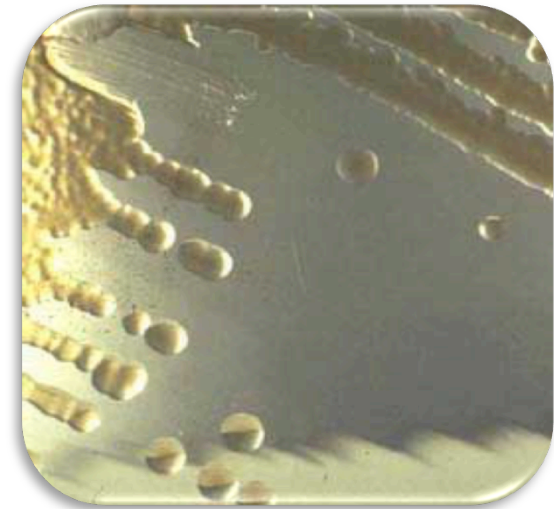
### ■ Rectal swab

### ■ Culture

- Enrichment in alkaline peptone broth + 1-3% NaCl
- Selective media, such as thiosulfate citrate bile-salts (TCBS) agar (also useful for *V.parahaemolyticus*)

### ■ Identification

- MALDI-TOF MS, biochemical characters → *V.cholerae*
- Serologic typing → *V.cholerae* O1, O139 or other
- Detection of toxine (PCR) if O1 or O139 → « Cholera »



## Confirmation methods proposed by the Belgian National Reference Center (NRC) for *Vibrio* when a suspected case is encountered

**Sacheli Rosalie, PhD**

National Reference Center for *Vibrio cholerae* and *parahaemolyticus*-Clinical Microbiology unit- CHU of Liège

- **Les sérogroupes pathogènes produisent la toxine cholérique, alors que les souches non pathogènes peuvent ou non produire cette toxine.**
- **Le bactériophage filamenteux Ctx confère aux souches de *Vibrio cholerae* (*V. cholerae*), l'opéron Ctx, portant les gènes CtxA et CtxB.**