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 deaths 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
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

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
    - Fecal 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 µm to 3 µm
- 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

Serogp O1

Non-O1; O139 & other serogp

Serotypes

Biotypes

Classique

El Tor

Ogawa

Inaba

Hikojima

Other species

V. parahaemolyticus (Foodborn diarrhea)

V. vulnificus (Wound infection, bacteriemia)

V. alginolyticus (external ear and wound infection)
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 **enterotoxin**
    - 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 \(\downarrow\) 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)
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
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, azythromycin)
    - To reduce length of illness
      → reduction of time of excretion
      → reduction of volume of rehydration solution
New formula meets WHO requirements

Each sachet contains the equivalent of:
- Sodium Chloride: 2.6 g
- Potassium Chloride: 1.5 g
- Tribasic Sodium Citrate, dehydrate: 2.9 g
- Glucose Anhydrous: 13.5 g

DIRECTIONS
Dissolve in ONE LITER of drinking water.

To Be Taken Orally according to age or as otherwise directed under medical supervision.
- Infants: over a 24 hour period.
- Children: over an 8 to 24 hour period.

CAUTION: DO NOT BOIL SOLUTION

Distributed by: AGS Brands, INC
4871 Sharp Street • Dallas, TX 75247
1-800-531-6731 • www.agsbrands.com
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.
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
STOP CHOLERA
it’s in your hands!

PROTECT YOURSELF

DRINK CHLORINATED OR
BOILED WATER

WASH HANDS WITH SOAP AFTER
USING THE TOILET AND BEFORE EATING

ALWAYS USE A LATRINE OR TOILET

EAT HOT, COOKED FOOD

WASH FRUITS AND PLATES
ON CHLORINATED WATER

MEDECINS SANS FRONTIERES
Poster éducatif, en créole haïtien, pour lutter contre la diffusion du choléra

Pwoteje têt nou pou n pa trape kolera

Source : Ministère de la santé publique et de la population (MSPP) / Dinepa / Unicef / ACF
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
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
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 seaproducys
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

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

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*).
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.

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

*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**
    - *A fecal stool « eau de riz »*
    - Under microscope
      - No leucocytes
      - Monomorphic flora: curved bacilli
  - **Rectal swab**

- **Culture**
  - Enrichment in alcaline 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 *Vibrion cholerae (V. cholerae)*, l’opéron Ctx, portant les gènes CtxA et CtxB.