



*Vibrio* spp.



12/04/2020

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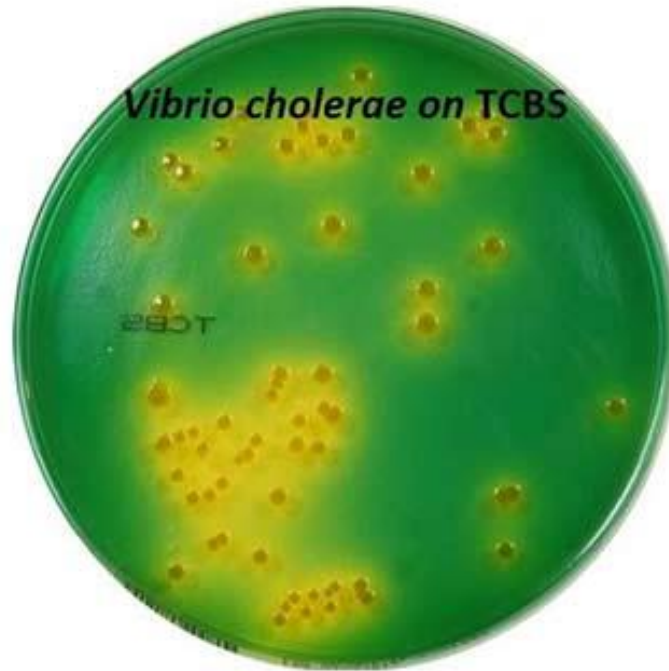
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- *Vibrio* spp.
- Growth conditions, dissemination, disease
- *Vibrio parahaemolyticus*
- *Vibrio cholera* Serogroups O1 and O139
- *Vibrio vulnificus*
- Virulence factor
- Pathogenesis

## *Vibrio spp.*

- Gram-negative, straight or curved rods.
- Motile by monotrichous (polar) flagellum.
- Occur naturally in aquatic environments (freshwater to open ocean).
- Highly susceptible to low pH, freezing, and cooking.
- Pathogenic species:
  - *Vibrio cholerae* serogroups O1 and O139 – Gastroenteritis
  - *Vibrio parahaemolyticus* – Gastroenteritis, wound infection, bacteremia
  - *Vibrio vulnificus* – Bacteremia, wound infection (high fatality)





*Vibrio cholerae* on TCBS Agar

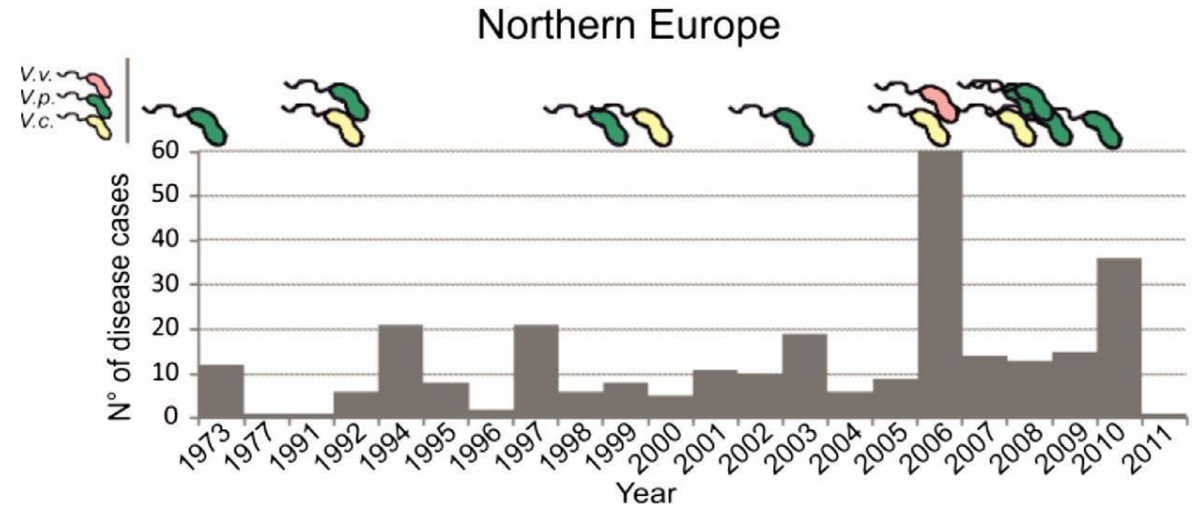
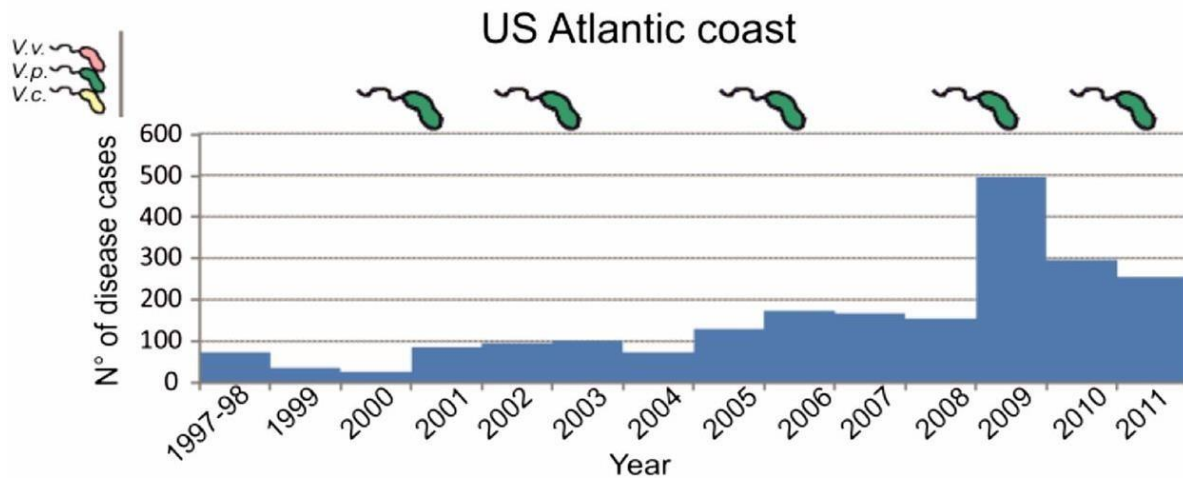
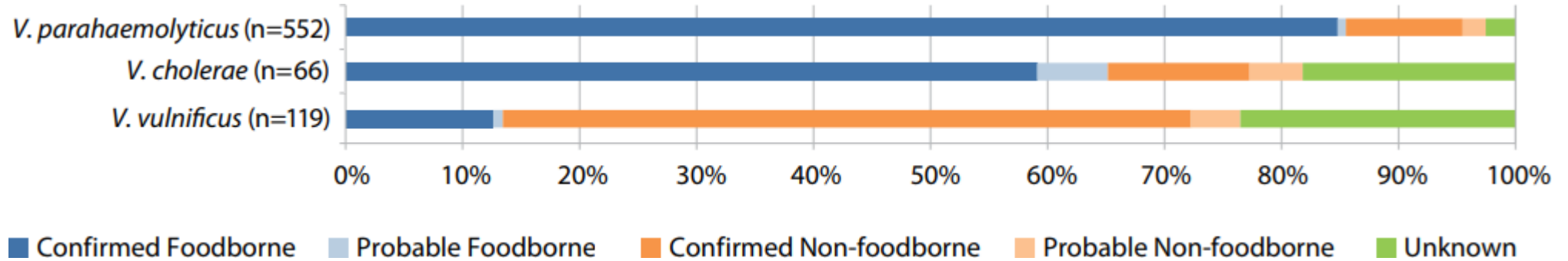



*Vibrio parahaemolyticus* on TCBS Agar

## TCBS agar

- pH indicator (bromthymol blue & thymol blue) turn yellow – sucrose fermentation
- Sodium chloride provide optimum growth and metabolic activity of halophilic *Vibrio* spp.

# VIBRIOSIS



A top-down view of a platter of oysters on the half shell. The oysters are arranged on a bed of crushed ice. Several bright yellow lemon wedges are scattered among the oysters. The platter is set on a light blue wooden surface.

## WHY *V. parahaemolyticus* MOSTLY LINKED TO OYSTER?

OYSTER  
CONCENTRATE THE  
BACTERIUM 100X  
THAN WATER

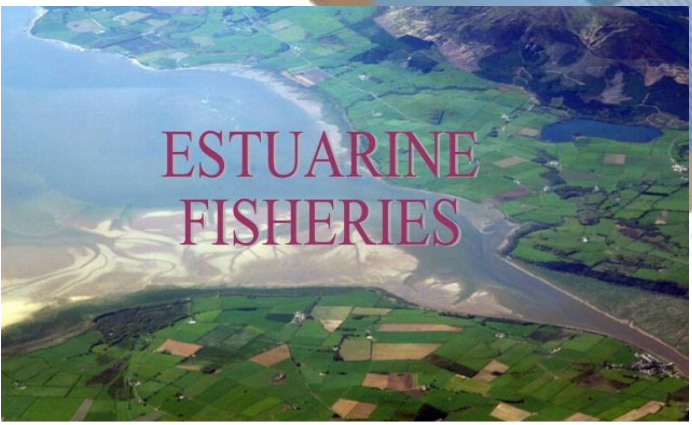
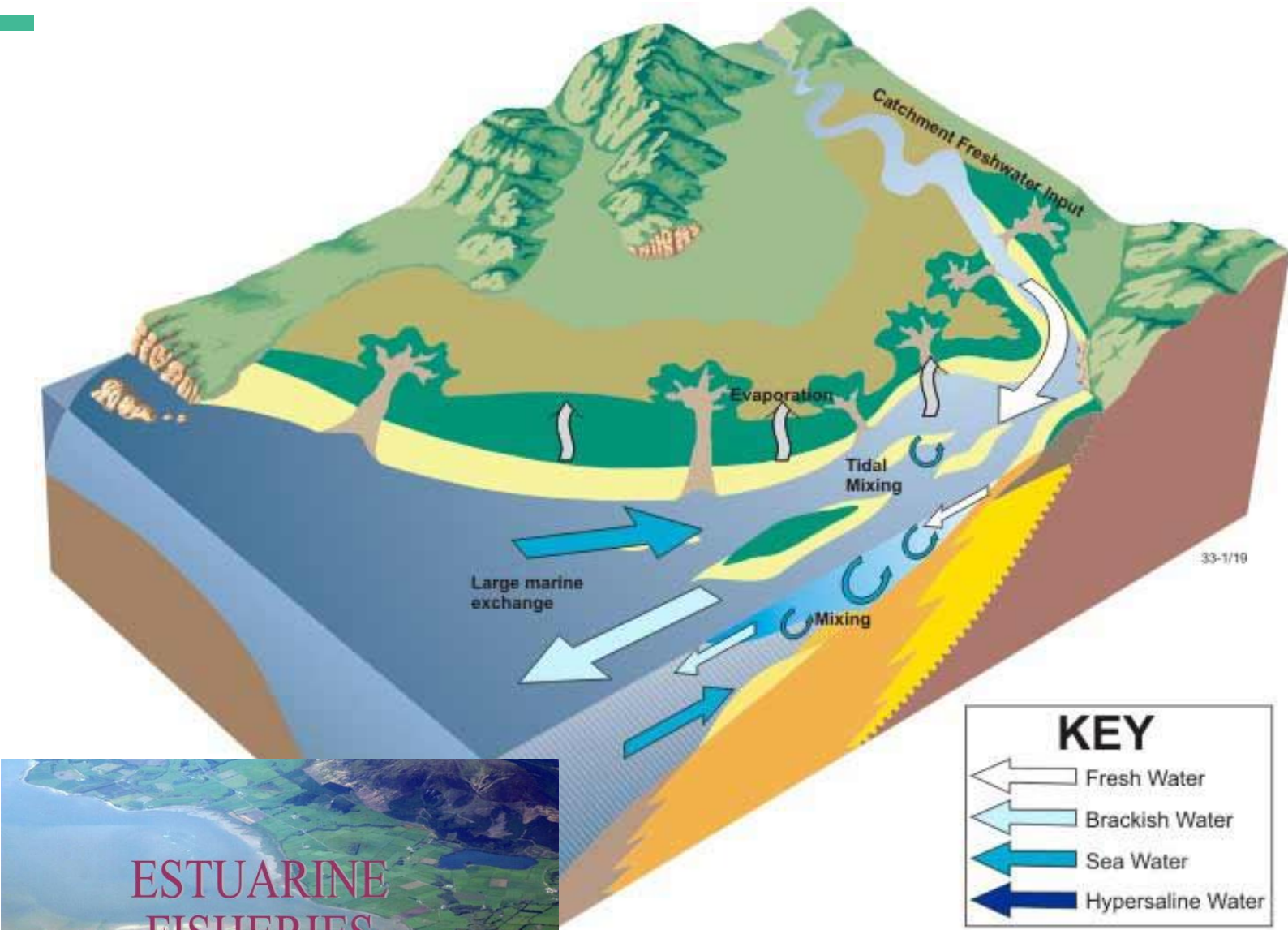
AS COMPARISON FECAL  
COLIFORM IS 10X  
GREATER IN OYSTER  
THAN WATER

# *Vibrio parahaemolyticus*

- It has a single flagellum
- Classification is based on somatic (O) antigen and capsular (K) antigen patterns.
  - Predominant serotype O3:K6, O4K12, O4:K68
- Most species isolated is non-pathogenic.
- Pathogenic strains produce **hemolysins** in host cells – cause lysis of red blood cells & affect electrolyte balance.
- Lysed almost immediately in freshwater; thus, it is not usually transmitted via the fecal-oral route.



- Occur naturally in warm coastal areas (estuarine and marine)
- Temperate countries experience higher numbers of outbreak during summer while tropical countries are conducive for year-round outbreaks.
- Global climate change increase in the distribution worldwide



Click on image for notes on Hydrodynamic Processes



# GROWTH CONDITIONS

- **Mesophile** (optimum: 20 - 35 °C)
- Grow at min 15°C and max at 44°C
- Slowly inactivated at temperatures <10°C.
- **Halophilic**: highest abundance in oysters (23% salt).
- At least requires 0.5% NaCl (Optimal: 2% salt)
- Highly susceptible to low pH, freezing, and cooking.
- Can grow in foods that have been cooked, but have then been contaminated by raw food.

# DISSEMINATION

- Route of entry:
  - Oral (gastroenteritis infections)
  - Direct exposure (wound infections)
- Consumption of **raw/improperly cooked seafoods**
- **Summer** time between May and October (water  $T > 15^{\circ}\text{C}$ )
- **Filter-feeding shellfish** accumulate *V. parahaemolyticus* in their meat (oyster, squid, octopus, shrimp, crab, clams)
- Survival in freshwater is associated to adherence to algae.
- US: about 45,000 illnesses each year (86% foodborne)



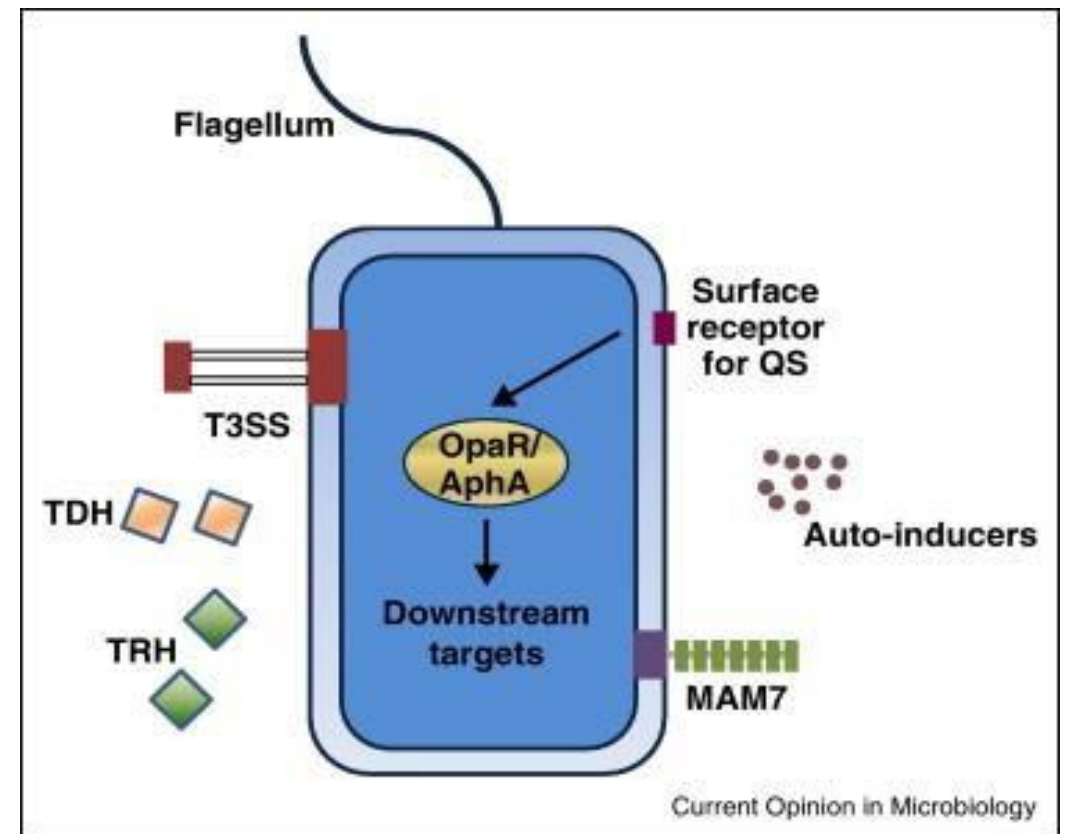
# DISEASE

- Infective dose:  $10^5$  -  $10^8$
- Onset: 4 to 90 hours incubation period, average 17 h
- Duration: 2 – 6 d
- Symptoms: mild to moderate gastroenteritis (watery/bloody diarrhea, stomach cramps, fever, nausea, and/or vomiting). Less than 40% of reported cases requiring hospitalization or antibiotic treatment.
- **Septicemia** in susceptible & people under medication (diabetes, liver disease, kidney disease, cancer, AIDS).
- Mortality: 2% of gastroenteritis, 20-30% of septicemia cases.

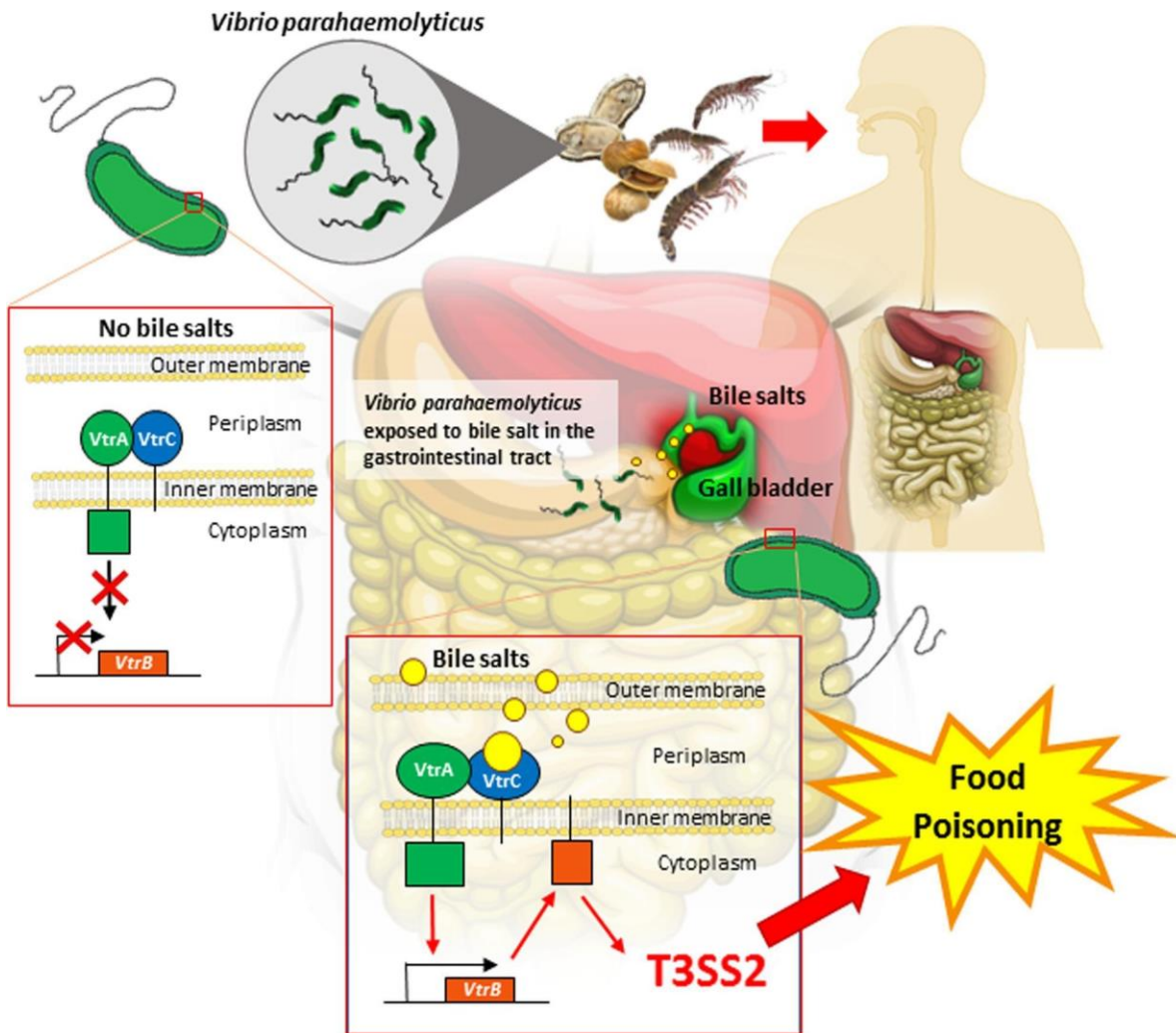
# VIRULENCE FACTORS

- **Adhesion:** use **adhesin MAM7** to attach to the eukaryotic cells.
- **Toxins:** cause hemolysis & cytotoxic activity
  - **TDH** (thermostable **direct** hemolysin)
  - **TRH** (heat-labile TDH-related hemolysin)
- TDH and TRH are **pore-forming toxin** that lyses red blood cells.
- The large pore sizes enable water and ions to flow through the membrane & disrupting **electrolyte balance**. → diarrhea

- 2 sets of **Type 3 secretion system** (T3SSI & T3SS2) - Inject virulence proteins inside the host cells to allow bacterial **invasion** and **replication**



# How *Vibrio parahaemolyticus* sense bile salts in the human gastrointestinal tract and release toxins??



- Bile is the fluid made by the liver, stored in the gallbladder & sent to the intestine for digestion of fats.
  - Bile salts help to digest fats (break down larger fat globules in food into small droplets of fat)
- *V. parahaemolyticus* from contaminated seafoods is ingested and exposed to bile salts in the GI tract.
- Bile salt triggers the expression of virulence genes via the **Type three secretion system 2** (encoded in the pathogenicity island Vp-PAI).
  - When *V. parahaemolyticus* is exposed to bile salts, *VtrA* and *VtrC* genes were triggered & form VtrA/VtrC protein complex that binds to bile salts.
  - The activation of VtrA induces VtrB to be activated.
  - The toxins production is triggered when VtrB activate T3SS2 expression.
  - T3SS2 virulence is secreted thus causing illness to human.

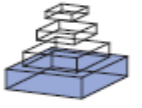
Letchumanan et al. 2017,

<https://doi.org/10.3389/fmicb.2017.00728>



# OUTBREAKS

- 2018: Multistate Outbreak of *Vibrio parahaemolyticus* Infections Linked to Fresh Crab Meat Imported from Venezuela
- 2017: 145 cases in Maryland, 25 cases in Seattle, 7 cases in Alabama - gastroenteritis & serious bacterial skin infection
- 2015: 70 cases in Canada due to raw shellfish, primarily oysters.
- 2003: 12 provinces in China, 2795 foodborne disease outbreaks, 62,559 illnesses, 31,261 hospitalizations, and 330 deaths.



# Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia

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57.8% isolates (185/320) were positive for *V. parahaemolyticus* in shrimps purchased from wet markets and supermarkets

*Vibrio parahaemolyticus* is a marine and estuarine bacterium that has been the leading cause of foodborne outbreaks which leads to a significant threat to human health worldwide. Consumption of seafood contaminated with *V. parahaemolyticus* causes acute gastroenteritis in individuals. The bacterium poses two main virulence factors including the thermostable direct hemolysin (*tdh*) which is a pore-forming protein that contributes to the invasiveness of the bacterium in humans and TDH-related hemolysin (*trh*), which plays a similar role as *tdh* in the disease pathogenesis. This study aimed to investigate the antimicrobial resistance of *V. parahaemolyticus* strains in shrimps purchased from wetmarkets and supermarkets. The *toxR*-based PCR assay indicated that a total of 57.8% (185/320) isolates were positive for *V. parahaemolyticus*. Only 10% (19/185) *toxR*-positive isolate exhibit the *trh* gene and none of the isolates were tested positive for *tdh*. The MAR index was measured for 14 common antimicrobial agents. The results indicated 98% of the isolates were highly susceptible to imipenem, ampicillin sulbactam (96%), chloramphenicol (95%), trimethoprim-sulfamethoxazole (93%), gentamicin (85%), levofloxacin (83%), and tetracycline (82%). The chloramphenicol (*catA2*) and kanamycin (*aphA-3*) resistance genes were detected in the resistant *V. parahaemolyticus* isolates. Our results demonstrate that

# Prevalence and Antimicrobial Susceptibility of *Vibrio parahaemolyticus* Isolated from Short Mackerels (*Rastrelliger brachysoma*) in Malaysia

Out of 130 short mackerel samples, 116 (89.2%) were detected with the presence of total *V. parahaemolyticus*

Chia W. Tan<sup>1\*</sup>, Tan T. H. Malcolm<sup>1</sup>, Chee H. Kuan<sup>1</sup>, Tze Y. Thung<sup>1</sup>, Wei S. Chang<sup>1</sup>, Yuet Y. Loo<sup>1</sup>, Jayasekara M. K. J. K. Premarathne<sup>1,2</sup>, Othman B. Ramzi<sup>1</sup>, Mohd F. S. Norshafawatie<sup>1</sup>, Nordin Yusralimuna<sup>1</sup>, Yaya Rukayadi<sup>1</sup>, Yoshitsugu Nakaguchi<sup>3</sup>, Mitsuaki Nishibuchi<sup>1</sup> and Son Radu<sup>1,4</sup>

<sup>1</sup> Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Malaysia, <http://journal.frontiersin.org/article/10.3389/fmicb.2017.01087/abstract>  
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## OPEN ACCESS

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Numerous prevalence studies and outbreaks of *Vibrio parahaemolyticus* infection have been extensively reported in shellfish and crustaceans. Information on the quantitative detection of *V. parahaemolyticus* in finfish species is limited. In this study, short mackerels (*Rastrelliger brachysoma*) obtained from different retail marketplaces were monitored with the presence of total and pathogenic strains of *V. parahaemolyticus*. Out of 130 short mackerel samples, 116 (89.2%) were detected with the presence of total *V. parahaemolyticus* and microbial loads of total *V. parahaemolyticus* ranging from



# *Vibrio cholera* serogroups O1 and O139

- Most hardy of pathogenic of *Vibrio* spp. It has the potential to kill within hours.
- Ability to survive in **freshwater** and in water composed of up to ~3% salt.
- The most common source of transmission is through ingestion of food and **water** that has been **contaminated with fecal matter** from cholera- infected persons found in inadequate water treatment & poor sanitation.
- Produce **cholera toxin (CT)** and **toxin co-regulated pilus (TCP)**.
- Originated from **reservoir** in the **Ganges delta** in India, Cholera is now endemic in many countries.



# Source of contamination

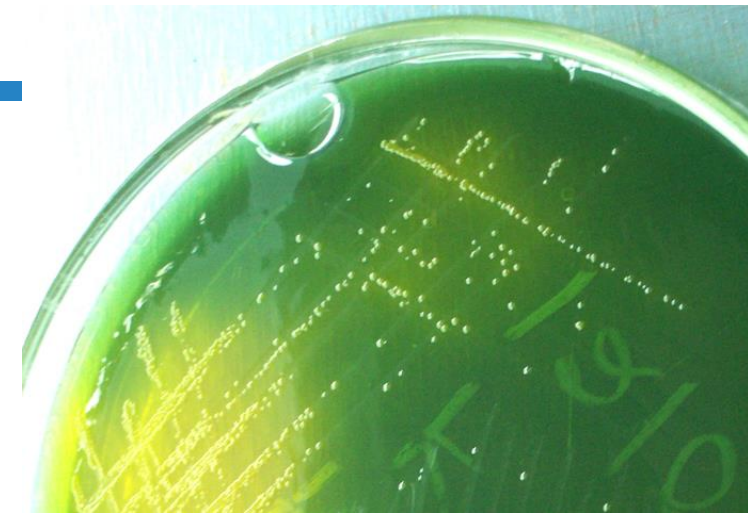


This waste water drainage was the suspected source of contamination of the well water that led to the cholera outbreak in Nigeria, April 2014. Two samples collected from home wells around this drainage tested positive for *Vibrio Cholerae*.



# CHARACTERISTICS

- Very susceptible to disinfectants, cold temperatures (especially freezing), and acidic environments.
- They are readily inactivated at  $T > 45^{\circ}\text{C}$ , and cooking food is lethal to *V. cholerae* O1 and O139.
- *V. cholerae* O139 is unique among that it is **encapsulated**.
- Most people infected with *V. cholerae* **do not develop any symptoms**, although the bacteria are present in their faeces for 1-10 days after infection and are shed back into the environment, potentially infecting other people.



# DISEASE

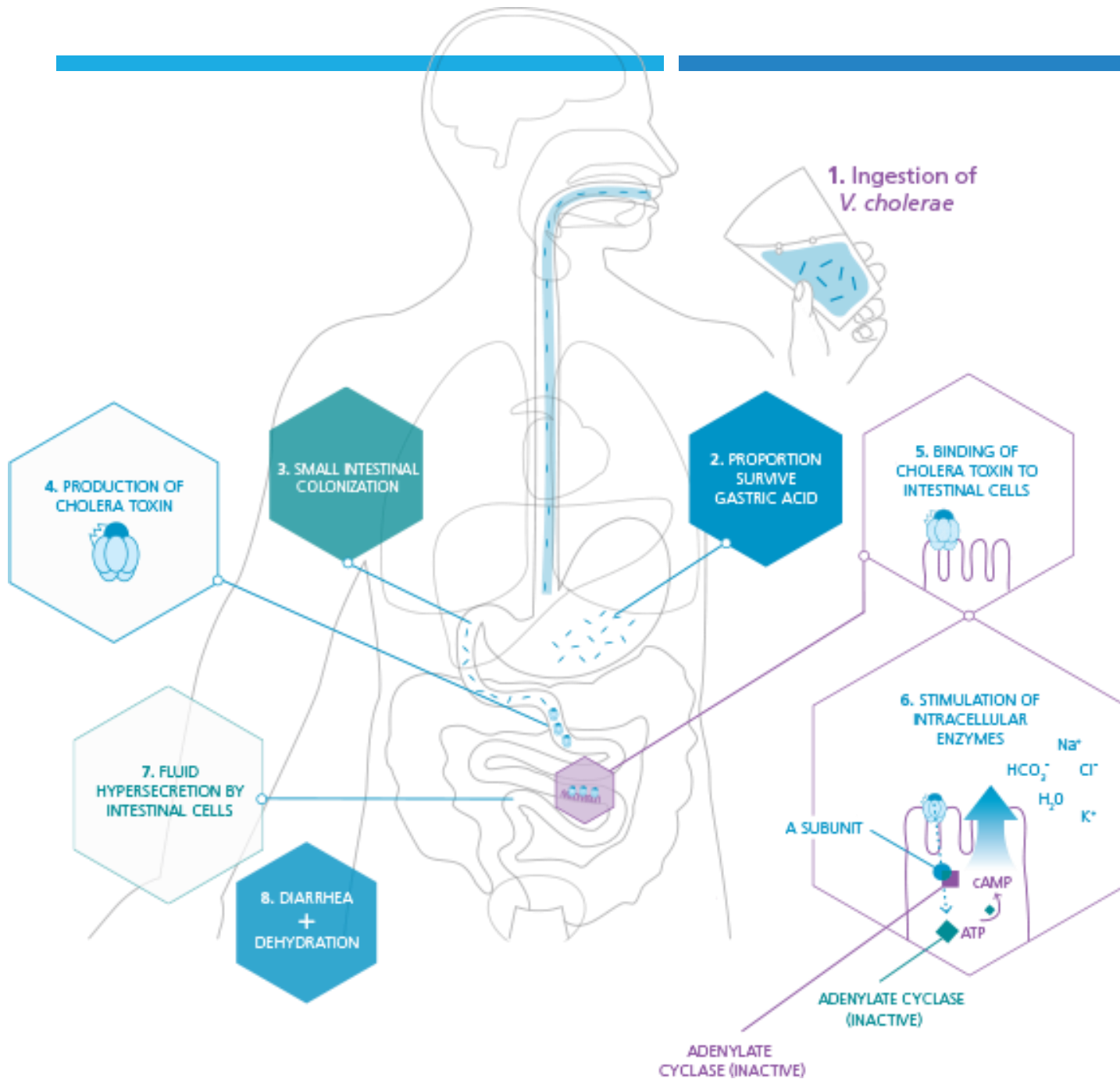
- Infective dose:  $10^6$
- Onset: **few hours** to 4 days of ingestion
- Duration: **few days** (depending on the severity of illness when treatment is initiated)
- Symptoms: mild to severe diarrhea (20% watery diarrhea, 10-20% of those develop **severe watery diarrhea** (characteristic **rice-water stools** and vomiting)
- Mortality: 30% to 50%, timely treatment, the fatality rate is <1%.
- Treatment: oral rehydration solution (ORS), IV fluid treatment for severe cases, antibiotic
- Prevention: Cholera vaccine, wastewater treatment, good hygiene and sanitation practice, disease surveillance,

# TREATMENT

- Cholera is an **easily treatable disease**.
- The majority of people are successfully treated through prompt administration of **oral rehydration solution (ORS)**.
- WHO/UNICEF ORS standard sachet is dissolved in 1 L of clean water. Adult patients may require up to **6 L of ORS** to treat moderate dehydration on the first day.
- Severely dehydrated patients are at **risk of shock** and require the rapid administration of intravenous fluids. **Appropriate antibiotics** is also given to diminish the duration of diarrhea and reduce the volume of rehydration fluids needed,.
- **Zinc** is an important adjunctive therapy for children under 5, which also reduces the duration of diarrhoea.

# TOXICO-INFECTION MECHANISM

- Pathogenesis *V. cholerae* critically depends on the cholera toxin and toxin-coregulated pilus (TCP).
- *V. cholerae* toxin **stimulates intracellular enzymes** that increase **Cl<sup>-</sup> secretion** by intestinal cells & **inhibition of Na<sup>+</sup> and Cl<sup>-</sup> absorption** by the microvilli.
- Massive purging of **electrolyte-rich fluid** into the **small intestine** exceeds the absorptive capacity of the colon.
- cause rapid dehydration and depletion of electrolytes (Na<sup>+</sup>, Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, K<sup>+</sup>).





Youtube: Cholera toxin mode of action

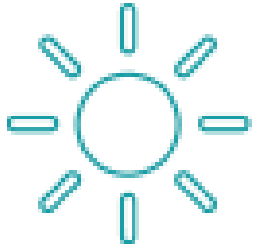
<https://youtu.be/QDp7a8yIHpc>

Reading materials:

WHO (2017) **Cholera**. <http://www.who.int/mediacentre/factsheets/fs107/en/>



A person with cholera can pass up to **20 LITERS OF DIARRHEA** in a single day.<sup>20</sup>



An American physician working in Pakistan documented his experience after developing cholera<sup>24</sup>:

## CHOLERA BY THE HOUR

**8 AM** Acute onset of **DIARRHEA**; diffuse abdominal cramps; anorexia

**9 AM** Oral rehydration and **ANTIBIOTICS** started

**11 AM** Light-headedness and **NAUSEA**

**12 PM** **VOMITING**; Blood pressure 80/60 mm Hg and pulse rate 116/min

**12:20 PM** Persistent severe **MUSCLE CRAMPS** in both lower legs

**2 PM** Rice-water stool while en route to **HOSPITAL**

**5 PM** (at hospital): 8.8 lb **WEIGHT LOSS**

**7 Cholera PANDEMICS** »  
HAVE OCCURRED  
since **1816**<sup>6,7</sup>

.....  
The seventh and current  
pandemic has persisted  
for more than 50 years

- 1<sup>ST</sup> 1816-1826
- 2<sup>ND</sup> 1829-1851
- 3<sup>RD</sup> 1852-1860
- 4<sup>TH</sup> 1863-1875
- 5<sup>TH</sup> 1881-1896
- 6<sup>TH</sup> 1899-1923
- 7<sup>TH</sup> 1961-Present

**2 US PRESIDENTS**  
DIED FROM CHOLERA<sup>8</sup>

.....  
**JAMES POLK 1849**

**ZACHARY TAYLOR**  
**1850**



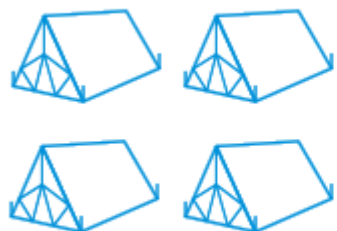
**1992 OUTBREAK**  
FROM A FLIGHT<sup>11</sup>

.....  
Largest outbreak of cholera ever associated  
with a commercial flight traveling from Buenos  
Aires, Argentina, to Los Angeles, California

**100** PASSENGERS INFECTED    **10** HOSPITALIZED  
**75** REPORTED DIARRHEA    **1** DIED

**Original reservoir in the Ganges delta in India.**

**2010 HAITI OUTBREAK**  
FOLLOWING EARTHQUAKE<sup>12,13</sup>



.....  
Largest cholera outbreak  
in recent history after an  
earthquake in Haiti

**735,000** INFECTED  
**9,000** DEATHS

**2015**  
AND **ONGOING**  
OUTBREAK IN TANZANIA<sup>15</sup>

**>4,400** AFFECTED  
**68** DEATHS



# 2010 HAITI OUTBREAK FOLLOWING EARTHQUAKE<sup>12,13</sup>



Largest cholera outbreak  
in recent history after an  
earthquake in Haiti



**735,000** INFECTED  
**9,000** DEATHS



## Case study: 2010 cholera outbreak in Haiti

- 2 hypothesis:
  - I. climatic hypothesis – nonpathogenic *Vibrio cholerae* evolved into a pathogenic strain.
  - II. human transmission hypothesis – cholera was introduced to Haiti by individuals infected in a foreign country.





# HOW A SARI CAN SAVE A LIFE

- In 1950s, Dr Rita Cowell saved 50 villages in Bangladesh from cholera endemic by using sari.

“If you provide safer drinking water, it does a tremendous job of preventing the spread of disease.”

**Dr. Rita Colwell**  
2010 Stockholm Water Prize Laureate

#StockholmWaterPrize Siwi.org/prizes


# *Vibrio vulnificus*

- Similar characteristics to *V. parahaemolyticus*
- Halophilic: highest abundance in oysters (23% salt).
- At least requires 0.5% NaCl (Optimal: 2% salt)
- Lysed almost immediately in freshwater; thus, it is not usually transmitted via the fecal-oral route.
- Contaminated water affect seafood
- Under 100 cases a year. But the illness is more often **fatal**.

# GROWTH

- Mesophile (optimum: 20 - 35 °C)
- Grow up to 41 °C
- Slowly inactivated at temperatures <10°C.
- Highly susceptible to low pH, freezing, and cooking.
- Can grow in foods that have been cooked, but have then been contaminated by raw food.

# DISEASE

- Infective dose:  $10^6$
- Onset: 12 hours to 21 days
- Duration: gastroenteritis is self-limiting. The mean duration of septic illness is 1.6 days.
- Symptoms: fever (in the case of septicemia), diarrhea, abdominal cramps, nausea, and vomiting.
- In early detection certain antibiotics can be used to treat it.
- People with weak immune system illness *Vibrio* can **infect the blood** and cause serious infections in other parts of the body.
- Mortality: 35% of septicemia, 20% of wound-infection

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# VIRULENCE FACTORS

- *V. vulnificus* harbors many putative virulence factors.
- Including capsule, pili, hemolysins, metalloproteases, and enterotoxins.
- However, none of these factors has been shown unequivocally to be essential in causing disease; much remains unknown.



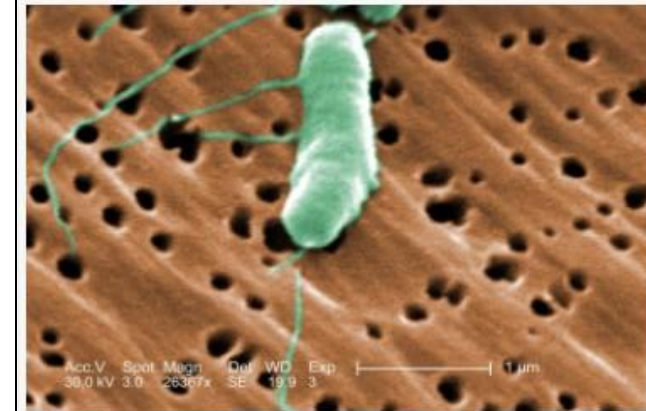
# Vibrio vulnificus deaths reported in Monroe, St. Johns counties as state case count tops 40

by ROBERT HERRIMAN

October 13, 2017 US News No Comments

2901 views

In a **follow-up on the Vibrio vulnificus situation in Florida in 2017**, eight additional cases and two deaths have been reported in the past two weeks.



Vibrio vulnificus/CDC

According to Florida Department of Health data, as of Oct. 13, 41 cases have been reported from 20 counties. In addition, two additional fatalities have been reported- one each in St. Johns and Monroe Counties.

Last year, Florida reported 46 cases, including 10 deaths.

The Centers for Disease Control and Prevention (CDC) says *Vibrio vulnificus* is a bacterium in the same family as those that cause cholera and *Vibrio parahaemolyticus*. It normally lives in warm seawater and is part of a

group of vibrios that are called “halophilic” because they require salt.

## Related: Florida reports 1st locally acquired Zika case of year in Manatee County

*V. vulnificus* can cause disease in those who eat contaminated seafood or have an open wound that is exposed to seawater. Among healthy people, ingestion of *V. vulnificus* can cause vomiting, diarrhea, and abdominal pain. In immunocompromised persons, particularly those with chronic liver disease, *V. vulnificus* can infect the bloodstream, causing a severe and life-threatening illness characterized by fever and chills, decreased blood pressure (septic shock), and blistering skin lesions. *V. vulnificus* bloodstream infections are fatal about 50% of the time.

*V. vulnificus* can cause an infection of the skin when open wounds are exposed to warm seawater; these infections may lead to skin breakdown and ulceration. Persons who are immunocompromised are at higher risk for invasion of the organism into the bloodstream and potentially fatal complications.

# Vibrio vulnificus: Florida Reports Eight Cases, Two Deaths

Jun 19, 2015 | Emily Cohn | Outbreak News



by CC Image Courtesy of James Willamor on Flickr

Health officials in Florida have reported eight cases of *Vibrio vulnificus* infections, including two deaths, thus far in 2015. The cases were reported across seven counties and occurred on both the Gulf and Atlantic coasts [1]. As we enter the summer months, the 2015 case count appears to be on par with past years. In 2014, Florida reported a total of 32 *Vibrio vulnificus* infections, seven of which resulted in death [2]. *Vibrio vulnificus* infections are not isolated to Florida; cases have been reported from all of the states bordering the Gulf of Mexico [3]. The Centers for Disease Control (CDC) reports an average of 50 *Vibrio vulnificus* cases from the Gulf Coast Region each year, and a national average of up to 96 cases per year [3].

# *Vibrio vulnificus* & Wounds

[Español \(Spanish\)](#)

You may have heard that you can get *Vibrio* infection from eating raw or undercooked oysters and other seafood. But did you know you can also get a *Vibrio* infection through an open wound? This can happen when a wound comes into contact with raw or undercooked seafood, its juices, or its drippings or with saltwater or [brackish water](#).\*

One species, *Vibrio vulnificus*, can cause life-threatening wound infections. Many people with *Vibrio vulnificus* infection require intensive care or limb amputations, and about 1 in 5 people with this infection die, sometimes within a day or two of becoming ill.

Some *Vibrio vulnificus* infections lead to [necrotizing fasciitis](#), a severe infection in which the flesh around an open wound dies. Some media reports call this kind of infection "[flesh-eating bacteria](#)," even though necrotizing fasciitis can be caused by more than one type of bacteria.

## What are the signs and symptoms of *Vibrio vulnificus* infection?

Signs and symptoms of *Vibrio vulnificus* infection can include:

- Watery diarrhea, often accompanied by stomach cramping, nausea, vomiting, and fever
- For bloodstream infection: fever, chills, dangerously low blood pressure, and blistering skin lesions
- For wound infection, which may spread to the rest of the body: fever, redness, pain, swelling, warmth, discoloration, and discharge (leaking fluids).

## How is infection diagnosed and treated?

Infection is diagnosed when *Vibrio* bacteria are found in the wound, blood, or stool (poop) of an ill person. The infection is treated with antibiotics. Doctors may need to amputate a patient's legs or arms to remove dead or infected tissue.



Thank  
you

