

# CASE 1: CHOLERA


## Microbiology Laboratory Questions



# Travelling in India

## Scenario



 Fulfilling a long held travel dream, Robert has taken six months off work and is making his way through India taking in the sights, experiencing local festivals and making time to get to know the people. He is cautious in his hygiene, eating and drinking habits but despite this he contracts a **diarrhea with voluminous outpouring of fluid accompanied by vomiting**. He suspects **cholera** and with the help of a fellow traveler gets himself to a local hospital where a stool sample is examined and **his presumptive**

**diagnosis is confirmed**. He stocks up on appropriate fluids and stays put at the hostel he has booked into for a few days, experiencing some minor leg cramping along with the diarrhea. His curiosity about his illness has him reading up on the organisms when he returns to North America and **he is left wondering what serotype of Vibrio cholerae he might have contracted**, should he have been prescribed antibiotics, was there anything more he could have done to prevent contracting the organism and might he now be a carrier?

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**What are the other most common bacterial pathogens associated with this type of infectious scenario in Asia and in North America?**

# (i) Other possible bacterial causes

	<i>Bacteria: General Information</i>	<i>Cases per year: South Asia</i>	<i>Cases per year: North America</i>	<i>Common Symptoms</i>	
<b><i>Campylobacter jejuni</i></b>	<ul style="list-style-type: none"> <li>▪ Leading cause of foodborne illness</li> <li>▪ Found in poultry, raw meat, contaminated food and H2O</li> </ul>	20 million	1.3 million	Diarrhea Vomiting Abdominal pain/cramps Fever	Dysentery
<b><i>Shigella</i></b>	<ul style="list-style-type: none"> <li>▪ Gram-negative</li> <li>▪ 2<sup>nd</sup> most prevalent cause of foodborne disease</li> </ul>	19 million	500,000		Tenesmus Dehydration Dysentery Convulsions
<b><i>E. Coli</i></b>	<ul style="list-style-type: none"> <li>▪ Present in normal gut flora</li> <li>▪ Some pathogenic strains produce <b>Shiga toxin</b></li> </ul>	19 million	265,000		Stomach cramps
<b><i>Salmonella</i></b>	<ul style="list-style-type: none"> <li>▪ Found in raw meat, eggs, milk, manure</li> </ul>	4 million	1.2 million		Enteric fevers (life-threatening systemic illness)

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**What samples are taken for laboratory testing in these cases?**

**How important is the Microbiology Laboratory in the diagnosis of this particular infectious disease?**

## (ii) Laboratory testing & diagnosis

# Stool sample



### Diagnostic testing:

- Rules out other bacterial pathogens
- Determines exact **gastroenteric illness-causing pathogen**
- Allows for differentiation between normal gut microbiota and pathogenic microbes

### Importance of proper diagnosis:

- Necessary for prescribing **appropriate treatment** and intervention
  - Patient's symptoms are not unique to any single pathogen
    - Laboratory testing informs clinical team whether **antibiotics** should be prescribed

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**What are the tests that will be performed to detect the presence of any potential bacterial pathogens?**

# (iii) Microbiology laboratory tests

## Plating

- Plating cultures on selective & non-selective media
- Observe for presence of growth
- Colour of colonies

## ELISA

- Binding and fluorescing of antibodies to specific antigen

## Gram staining

- Gram negative bacteria: red
- Gram positive bacteria: purple
- Elucidate bacterial morphology

## PCR

- Important for confirmation of serotype





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**What are the expected results that allow for identification of the bacteria named in this case?**

## (iv) Expected results in the presence of *V. cholerae*

### Plating

- Translucent colonies with black centre, 1-2 mm in diameter on TTG agar
- Yellow colonies on TCBS agar

### ELISA

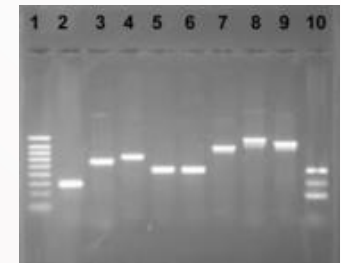
- Antibodies to cholera toxin (primary virulence factor) will fluoresce

### Gram staining

- Red stain (*V. cholerae* = gram negative bacteria)

### PCR

- Comparison of stool/bacterial isolates and strains of *V. cholerae* can confirm presence of pathogen

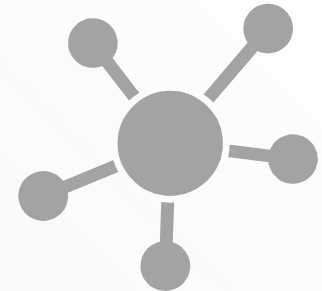


# Additional tests available

## Bacterial slide agglutination test

### *For serological identification:*

- *V. cholerae* suspension added to saline on slide
- Add **antiserum** specific to suspected serotype  
(e.g. *V. cholerae* O1)
- Expected results in presence of *V. cholerae* O1 antigen:
  - **Agglutination**



# Additional tests available

## Non-inhibitory Detection Methods

### Decarboxylase / dihydrolase reactions:

General Information	Expected result in presence of <i>V. cholerae</i>
<ul style="list-style-type: none"><li>▪ Purple = presence of <b>lysine decarboxylase</b></li><li>▪ Yellow = Presence of <b>arginine dihydrolase</b></li><li>▪ Can be used for <b>serotype identification</b></li></ul>	Purple for some serotypes only



A B C D

Some serotypes are positive for decarboxylase, while others are positive for dihydrolase.

A, C: decarboxylase +  
B: dihydrolase +  
D: control

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