



# CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

## Challenge M092-2

August 2009

Stool: *Vibrio cholerae* non O1, non O139

### HISTORY

The simulated stool sample was from a 37 year old pregnant woman, just returned from Mexico.

The sample was sent to category A and B laboratories and were requested to process and report as per their usual protocol. Participants were expected to process the sample according to their normal protocol, identify *Vibrio cholerae*, and report to the Public Health (PH).

### CMPT QA

The sample, verified at CMPT, yielded a 4+, pure culture of *V. cholerae*, viable for 14 days. This isolate was a non O1/non O139 strain of low pathogenicity.

### SURVEY RESULTS

This was the first *V. cholerae* challenge sent by CMPT.

#### Reference Laboratories:

14/15 labs reported *V. cholerae* +/- refer, 1 lab reported *Vibrio* species, refer. Consensus for identification was achieved by the reference labs thus the sample was considered suitable for grading.

Identification (see Table 1)

Seventy-two labs (66%) correctly identified *Vibrio cholerae* and received a grade of 4. Two of

the 72 labs (A labs) were able to perform serotyping on the strain and further identify it as non O1.

A total of 11 laboratories reported "*Vibrio* species", one suggested it could be *V. cholerae*; all indicated they would refer the sample for further testing and received a grade of 4.

One laboratory reported "*Aeromonas/Vibrio* species, refer" and received a grade of 3. Another laboratory reported that the microorganism was referred for *Vibrio* testing and was ungraded.

One laboratory reported "NSSYCEAP" and was graded zero and another laboratory reported "unknown organism, refer, NSSYCE" and received a grade of one.

A total of 22 (6% category A and 22% category B) laboratories do not normally process this type of sample and were ungraded.

Public Health notification (see Table 2)

The vast majority (96.5%) of those laboratories which identified or suspected *Vibrio* in the sample reported the findings to Public Health or Infection Control. These laboratories were given a grade of 4.

Two laboratories did not report despite isolating *Vibrio cholerae* from the sample and were graded zero. One laboratory reported "ID would be reported to the physician". This report was

### Grading

**Maximum grade: 8** (4 points each for identification and Public Health notification).

The ability of the laboratories to correctly identify the microorganism as *V. cholerae*, or as *Vibrio* species and to recognize the importance of submitting the isolate for further identification, was given the maximum grade (4).

Not processing the sample for *Vibrio*, *Aeromonas* or *Plesiomonas* species was considered unacceptable since these organisms are known enteric pathogens. Not detecting *V. cholerae*, when it is present in a stool sample, could have very serious epidemiological consequences.

Since cholera is an internationally notifiable disease, the public health authorities must be notified accordingly. Failure to do so, when *Vibrio* was suspected, was considered unacceptable and graded with a zero.

**Table -1:** Reported results for M092-2 -Identification component -

Reported	A	B	Total	%	Grade
<i>Vibrio cholerae</i> non-O1, presumptive, refer	2		2	1.8	4
<i>Vibrio cholerae</i> +/- presumptive, +/- refer, +/- NSSCEAP	63	7	70	64.2	4
<i>Vibrio</i> species, refer +/- presumptive, +/- NSSYCEAP	7	3	10	9.2	4
<i>Vibrio</i> species, possible <i>V. cholerae</i> , refer	1		1	0.9	4
<i>Aeromonas/Vibrio</i> species, refer		1	1	0.9	3
NSSYCE		1	1	0.9	0
unknown organism, refer, NSSYCE		1	1	0.9	1
referred for <i>Vibrio</i> testing, NSSYCE		1	1	0.9	ungraded
snp	5	17	22	20.2	ungraded
<b>Total</b>	<b>78</b>	<b>31</b>	<b>109</b>	<b>100.0</b>	

snp: sample not normally processed NSSYCEAP - no *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *E.coli* O157, *Aeromonas*, *Plesiomonas* isolated  
 NSSYCE - no *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *E.coli* O157 isolated

**Table –1:** Reported results for M092-2 –Public Health notification component

Reported	Total	%	Grade
Public Health notified	76	90.5	4
Infection Control Practitioner notified	5	6.0	4
n/a	24	n/a	ungraded
no report	2	2.4	0
see comment	1	1.2	0
<b>Total</b>	<b>109</b>	<b>100.0</b>	

considered unacceptable as the laboratory and the clinician both have a responsibility to report notifiable isolates.

## ISOLATION

In areas where *Vibrio cholerae* is not endemic special isolation media is not commonly used. Therefore, inclusion of pertinent clinical history should accompany specimens to alert the laboratory to include isolation techniques for *Vibrio* species in their stool workup. Examples of clinical history to be included are consumption of seafood, especially raw shellfish, significant exposure to marine or brackish water or associated wounds, and travel to endemic areas.

Since thiosulfate citrate bile salts sucrose agar (TCBS) does not require autoclave procedures, powdered media may be kept available in the laboratory and readily prepared by boiling whenever needed <sup>2</sup>.

TCBS is generally used for the isolation of *Vibrio* species associated with human disease.

Colonies suspicious for *V. cholerae* will appear on TCBS agar as yellow, shiny colonies, 2 to 4 mm in diameter. The yellow color is caused by the fermentation of sucrose in the medium. Sucrose-nonfermenting organisms, such as *V. mimicus* and *V. parahaemolyticus*, produce green to blue-green colonies <sup>3</sup>.

If enrichment is attempted, alkaline peptone water (APW) (1% NaCl, pH 8.5) is the most commonly used enrichment broth for human specimens. The stool inoculum should not exceed 10% of the volume of the broth; which is then incubated at 36°C. The surface and topmost portion of the broth is then subcultured at 6-18 hours, since *Vibrio* species preferentially grow on the surface of the broth. Longer incubation

times may fail to yield *Vibrio* species, probably due to overgrowth of other flora <sup>1 2</sup>.

## IDENTIFICATION

The genus *Vibrio* is composed of gram-negative, straight or curved rod-shaped bacteria, 0.5 to 0.8 µm wide and 1.5 and 3.0 µm long. All species in the genus are motile <sup>2 4</sup>.

Growth of all species is stimulated by sodium and is an absolute requirement of most *Vibrio* species. Generally *Vibrio* species are oxidase-positive <sup>2 4</sup>. All species grow at 20°C and the halophilic species usually require NaCl. Most media formulated with peptone and meat extracts contain enough salt for *V. cholerae* and *V. mimicus* to grow <sup>2 4</sup>. *Vibrio* species ferment glucose, but rarely produce gas. They also reduce nitrate to nitrite and grow on TCBS medium <sup>2</sup>.

Fermentative metabolism distinguishes members of the genus *Vibrio* from oxidase-positive *Pseudomonas* species. The production of oxidase and/or the salt requirement of *Vibrio* species differentiate them from the other members of Enterobacteriaceae.

It should be noted that oxidase testing is unreliable when performed directly on colonies growing on TCBS and should not be attempted.

Sensitivity to the vibriostatic compound O/129 is also useful in identifying members of the genus *Vibrio* although some strains isolated in India and Bangladesh have been shown to be resistant to the compound <sup>2</sup>. *Aeromonas* and *Plesiomonas* species are oxidase-positive, fermentative organisms that may initially resemble *Vibrio* species. *Aeromonas* species, however, are resistant to O/129, *Plesiomonas* species vary in sensitivity to O/129, and neither genus requires NaCl for growth <sup>4</sup>.

Phenotypically, the genus *Vibrio* can be divided

## Cholera Pandemics

1817: the first cholera pandemic began with spread of the disease outside the Indian subcontinent as far as southern Russia.

1826: second pandemic started and reached the major European cities by the early 1830s. In 1831, the pandemic reached the UK.

At that time cholera was thought to be spread by the “miasma” (like a fog) coming from the river, but the epidemiological study of John Snow in 1854 in London showed that the disease was associated with contaminated drinking water.

Three more pandemics, continuing up to 1925, involved Africa, Australia, Europe, and all the Americas.

The current (seventh) pandemic has involved almost the whole world. This pandemic began in Indonesia and the causative agent was a biotype of *V. cholerae* serogroup O1 called El Tor. It was first isolated in 1905 from Indonesian pilgrims travelling to Mecca at a quarantine station in the village of El Tor, Egypt.

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into the non-salt-requiring species, which includes *V. cholerae* and *V. mimicus* (Group 1), and the salt-requiring species, which includes the remainder of the pathogenic *Vibrio* species (Groups 2-6) <sup>2</sup>. *V. cholerae* ferments sucrose and is easily differentiated from *V. mimicus* by the ability of the first one to ferment sucrose.

Lysine decarboxylase (LDC) and arginine dihydrolase (ADH) activities can be used to further differentiate the pathogenic *Vibrio* species. *Vibrio parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus* are LDC-positive and ADH-negative, as are *V. cholerae* and *V. mimicus*. *Vibrio fluvialis*, *V. furnissii*, and *V. damsela* are ADH-positive, whereas *V. hollisae* is negative for both LDC and ADH activities. *Vibrio* species can be further differentiated based on the ability to ferment certain carbohydrates such as sucrose, lactose, and arabinose. *Vibrio metschnikovii* is distinguished from all the other pathogenic species of *Vibrio* by the lack of indophenol oxidase activity <sup>2,4</sup>.

Correct identification of *Vibrio* species by commercial identification systems is problematic at best, which can only correctly speciate 63 to 81% <sup>2,5</sup>.

*V. cholerae* is the most important species in the genus *Vibrio*. It has caused many cholera epidemics and millions of deaths. It is now divided into three major subgroups: *V. cholerae* O1, *V. cholerae* O139, and *V. cholerae* non-O1 depending on the ability to agglutinate with O1 antiserum.

*V. cholerae* non-O1 strains do not agglutinate in O1 or O139 antisera but are otherwise phenotypically identical to O1 and O139 *V. cholerae* strains in their biochemical reactions.

Cultures identified as *V. cholerae* should be sent to public health laboratories for O1 and O139 agglutination and cholera toxin testing. *V. cholerae* O1 isolates should be biotyped by the reference laboratory to determine if they are El Tor or classical biotypes.

#### Typing systems

Both O1 and non-O1 *V. cholerae* live in aquatic environments, which are their natural reservoirs, and can be introduced to humans through contamination of water and food <sup>6</sup>. The O antigen shows enormous serological diversity, with over 200 serogroups. Only the O1 and O139 serogroups cause epidemic and pandemic disease<sup>1</sup>. Serotyping is by far the most

widely utilized procedure. *V. cholerae* O1 and O139 antisera are commercially available.

Agglutination should be carried out by reference laboratories with subcultures onto non-selective medium, because colonies can autoagglutinate from TCBS medium, giving false-positive results.

Phage typing has been used for differentiating classical from El Tor biotypes, but is not commercially available and is limited to a few reference laboratories <sup>2</sup>.

### ANTIMICROBIAL SUSCEPTIBILITY

The resistance of *V. cholerae* to several antimicrobial drugs has been frequently reported in samples of clinical as well as of environmental origin in different endemic areas around the world <sup>7,8,9</sup>.

Most strains of *V. cholerae* are susceptible (>90%) in vitro to aminoglycosides, azithromycin, fluoroquinolones, tetracyclines, extended spectrum cephalosporins, carbapenems and monobactams <sup>10</sup>.

*Vibrio cholera* O1 El Tor and O139 strains from India and Bangladesh demonstrate moderate to high-level resistance to sulfamethoxazole, trimethoprim, and chloramphenicol <sup>2</sup>.

Continuous surveillance of antimicrobial resistance in *V. cholerae* needs to be performed in order to detect the emergence of multi-resistant strains.

CLSI has an interpretive guideline for *V. cholerae* antimicrobial testing and reporting, which is limited to ampicillin, tetracyclines, folate pathway inhibitors and chloramphenicol <sup>11</sup>.

### TREATMENT

Cholera is a readily treatable disease. The prompt administration of oral rehydration salts to replace lost fluids may result in cure. In especially severe cases, intravenous administration of fluids may be required to save the patient's life. Left untreated, however, cholera can kill quickly following the onset of symptoms <sup>16</sup>.

Tetracyclines are the most commonly-used antibiotic in the treatment of cholera. It reduces stool volume, duration of diarrhea, and duration of excretion of *Vibrio cholerae* to about half than that's seen in patients treated without antibiotics <sup>17</sup>.

(from page 1)

### Cholera Pandemics

In 1992, a newly described, non-O1 serogroup of *V. cholerae*, designated O139 Bengal, caused unusual cholera outbreaks in India and Bangladesh. Serogroups O139 Bengal and O1 now coexist and continue to cause large outbreaks of cholera in India and Bangladesh.

The O139 serogroup is likely to be the cause of the next (eighth) pandemic of cholera. In spring 2002, serotype O139 caused an estimated 30,000 cases in Dhaka, Bangladesh, exceeding the number of cases associated with El Tor. <sup>1</sup>

## CLINICAL RELEVANCE

Coastal waters are important reservoirs of *Vibrio cholerae*, and cholera is generally transmitted to humans via contaminated water or sea-food, including shellfish, crustaceans and fin-fish<sup>8</sup>. Chironomid (a widely distributed type of non-biting midge) egg masses have been shown to support the growth of *V. cholerae*<sup>19</sup>. Non O1 *V. cholerae* is widely distributed in coastal waters and is occasionally acquired in Canada.

The pathogenesis of cholera is related to the effect of cholera toxin. But there are other virulence factors which are important including a complex series of mediators that control toxin production and expression of pili that allow adherence to the gut wall. These are produced in response to environmental conditions in the gut. While the epidemic strains possess these virulence factors, the non O1, non O139 strains usually lack components or all of these virulence factors, although occasional strains are able to produce toxin.

Only *V. cholerae* O1 and O139 serotypes have been associated with cholera pandemics. The other serogroups, have not been associated with epidemics but have been associated with occasional outbreaks of cholera-like disease<sup>8</sup><sup>12</sup>. In 2003, non O1, non O139 *V. cholerae* strains in the stool specimens collected from patients in Kolkata, India, comprised 30.4% of the total cases that were microbiologically positive for *V. cholerae*<sup>12</sup>. Some serotypes of non O1 *V. cholerae* have been shown to produce cholera toxin.

Gastroenteritis caused by non O1/non O139 *V. cholerae* presents clinical symptoms such as diarrhea, abdominal cramps, nausea, vomiting, and fever. A strain of O75 caused infection in 8 patients in the southern United States between 2003 and 2007. Infection was associated with consumption of raw oysters and resulted in a cholera like illness. Although the strains were shown to produce cholera toxin, the production of other virulence factors was not indicated. Other isolates which have been shown to produce cholera toxin have been shown to belong to serotypes O141 (in the US and Japan), O49 and O8<sup>18</sup>.

Unlike *V. cholerae* serogroup O1 and O139, non O1/non O139 *V. cholerae* can lead to extraintestinal diseases, such as bacteremia, invasive soft tissue infections, and peritonitis<sup>13</sup>. Individuals with liver disease or an immunosuppressive condition are more vulnerable to *V.*

*cholerae* non O1 extraintestinal infections<sup>6</sup><sup>14</sup>. A review of non O1 non O139 *V. cholerae* infections in Taiwan during the 1990s, found that 15 episodes of bacteremia occurred, all in patients with hepatic cirrhosis, and 7 patients died. Many of the bacteremic patients had focal infections including spontaneous bacterial peritonitis and necrotizing fasciitis. In contrast, the patients with gastrointestinal infection had no underlying disease. The presentations of these infections was similar to that found with *V. vulnificus*.<sup>20</sup>

In developed countries, non-O1 *V. cholerae* is responsible for cholera-like but less severe watery diarrhea and blood, wound, ear, and respiratory tract infections. Cases of *V. cholerae* non-O1/non-O139 infections were documented in Austria. The cases were associated with recreational activities at the study area, the lake Neusiedler See, with cases of otitis and one lethal septicemia. The lake offers ideal conditions for *V. cholerae*, with moderate salinity and a high pH between 7.8 and 9.1<sup>15</sup>.

The committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or high workload.

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