EDUCATIONAL COMMENTARY - CAMPYLOBACTER

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**Florida licensees, please note: This exercise is NOT intended to fulfill your state education requirement for molecular pathology. It will fulfill requirements for microbiology.**

Learning Outcomes

On completion of this exercise, the participant should be able to

- describe the public health implications of campylobacteriosis;
- describe the characteristics of *Campylobacter* when cultured;
- list the 3 most common ways to detect *Campylobacter* in a stool sample; and
- describe the advantages and disadvantages of the various methods used to detect *Campylobacter*.

*Campylobacter* is one of the leading causes of foodborne illness in many developed nations. In 2015, there were over 6000 reported cases of *Campylobacter* infection in the United States. It is likely that the total illness rate is much higher due to many unreported cases. The Foodborne Diseases Active Surveillance Network (FoodNet), a program of the U.S. Centers for Disease Control and Prevention (CDC) in cooperation with other agencies, indicates that approximately 13 cases are diagnosed annually for every 100,000 persons in the population. Most *Campylobacter* infections are caused by consuming food contaminated with the microorganism, such as raw or undercooked poultry, food that has come in contact with uncooked poultry, contaminated water, or unpasteurized milk. People may also become infected by direct contact with infected animals. *Campylobacter* infection is rarely spread by human-to-human contact. Most infections are isolated events, with few widespread outbreaks. Fewer than 500 *Campylobacter* organisms can be enough to cause disease.

The associated disease, campylobacteriosis, usually manifests within 2 to 5 days of consuming contaminated food or water. Symptoms include diarrhea (often bloody), abdominal pain and cramps, fever, and vomiting. The disease may be more severe in young, elderly, or immunocompromised persons. It is usually self-limiting, lasting about a week. Most treatment is supportive, such as administering intravenous fluids to combat dehydration. In severe cases, antibiotics such as azithromycin or fluoroquinolones may be given to shorten the duration of symptoms. Unfortunately, antimicrobial resistance, especially to drugs in the fluoroquinolone class, is increasing, possibly due to the use of this class of drugs in poultry feed. Complications are rare, but include Guillain-Barré syndrome and reactive arthritis. In addition, the organism can cause infections outside of the gastrointestinal tract, in areas such as the gallbladder, abdominal cavity, heart, and central nervous system.
Campylobacter normally inhabits the gastrointestinal tract of many species of domestic mammals and fowl, including pigs and chickens. There are 17 to 18 species and 6 subspecies of the genus Campylobacter, with Campylobacter coli and Campylobacter jejuni being the species that cause most disease in humans. Symptoms of a Campylobacter-like illness in infants were first noted in 1886 by Theodor Escherich, a German-Austrian pediatrician, who termed the illness cholera infantum or “summer complaint.” The characteristics defining the genus Campylobacter were first described in 1963, but the actual organisms were not isolated in cultures until 1972.

**Laboratory Detection of Campylobacter**

**Culture**
The traditional method of detecting Campylobacter species is by culture. Campylobacter species are spiral-shaped, microaerophilic, thermophilic, gram-negative bacteria. Campylobacter is best isolated by incubating the sample at 42°C under microaerobic conditions with specialized media. Commercial products are available to create microaerobic atmospheres. Specialized media such as Campylobacter enrichment broth and selective agar media can improve the isolation and recovery of *C. jejuni* and *C. coli* from stool cultures. Selective media for culture of Campylobacter contains antibiotics such as trimethoprim, vancomycin, and polymyxin B to inhibit normal fecal flora and aid in isolating Campylobacter.

When grown on blood agar plates, Campylobacter has a grey-tan, moist-appearing colony. Presumptive Campylobacter species may be confirmed by microaerobic growth, positive oxidase test results, and a characteristic Gram stain showing thin, curvy (seagull) gram-negative bacilli. Colonies can be further identified by using the hippurate test to differentiate the two species that most often infect humans. *Campylobacter jejuni* is hippurate-positive and *C. coli* is hippurate-negative.

**Antigen Testing**
Direct antigen testing has become a common method of detecting Campylobacter species in stool samples. Direct antigen testing is easier to perform than culture and requires no special media or incubation. Depending on the test, the sample types include unpreserved stool or stool preserved in transport media. Two of the current Campylobacter antigen detection methods available are lateral flow and enzyme immunoassay.

Lateral flow immunoassays use monoclonal antibodies specific to an antigen shared by *C. jejuni* and *C. coli*. The sample is pipetted into the sample port of a device. The sample then migrates across a membrane that contains a monoclonal antibody conjugate which binds to the Campylobacter antigen if present within the sample, forming a complex. The sample migrates until it reaches the position where
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the *Campylobacter* antigen complex binds to immobilized campylobacter antibodies in the membrane, producing a visible line. The sample continues to migrate until it reaches the control zone where any excess monoclonal antibody conjugate will bind and produce a visible line (the control line). A visible line for the sample and control would indicate a positive result, whereas a visible line for the control only, would indicate a negative result.

In contrast with lateral flow immunoassays, enzyme immunoassays (EIA) use a solid surface called a microplate, with wells coated with polyclonal anti-*Campylobacter* Specific Antigen antibody. The sample is pipetted directly into these wells. If *Campylobacter* Specific Antigen is present in the sample, it will bind to the antibody in the microtiter well. The wells are incubated and then washed to remove any unbound material. An enzyme conjugate is added and the wells are incubated again. After incubation the wells are washed once again to remove any unbound conjugate. A substrate that binds with the conjugate is added, and a positive reaction is indicated by the development of color.

**Polymerase Chain Reaction (PCR)**

At one time, polymerase chain reaction (PCR) test methods were available to only a limited number of laboratories and were difficult to perform. Within the last few years, molecular methods have become widely available. Platforms include closed systems which yield rapid results with minimal hands-on time. Because testing is done one sample at a time for each unit, the throughput is low, which usually makes these systems unsuitable for laboratories doing high volumes of testing. Other platforms are open systems that can process many samples at the same time. This makes a system like this suitable for high-volume laboratories, but samples require extended hands-on time and have a turnaround time of approximately 5 hours.

**Method Advantages and Disadvantages**

**Culture**

Culture has long been considered the gold standard for isolating *Campylobacter*. However, *Campylobacter* is difficult to isolate, as it depends on specialized culturing procedures and is susceptible to environmental factors. In addition, culture practices vary greatly between institutions, with some facilities incubating the culture for only 24 hours instead of 48 to 72 hours, incubating at a temperature other than 42°C, and/or not using a system that will provide the correct microaerobic conditions. This variance may affect laboratories’ ability to recover the organism. Initiation of antibiotic therapy before the sample is collected may also decrease the ability of recovering the organism, increasing the chance of a false-negative culture. One advantage of using culture to isolate any organism is that the organism is readily available for antimicrobial susceptibility testing. With increasing antimicrobial resistance, this advantage should not be ignored. Using a transport medium such as Cary-Blair can enhance the ability to recover *Campylobacter* when transport times may delay culturing. Using broth enrichment can also
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increase the ability to recover *Campylobacter*. Turnaround time is generally 48 to 72 hours for a stool culture.

**Antigen Testing**
Antigen testing is rapid and easy to use, making it available for most laboratories. Depending on the test, sensitivity can vary from approximately 73% to 85% and specificity from 96% to 99%. Some authors have expressed concern that there may be a substantial number of false-positive results.

**Polymerase Chain Reaction (PCR)**
PCR is generally considered to be highly sensitive and extremely specific. Closed systems require little hands-on time and can generate results in 1 to 2 hours. With most closed systems, only one sample at a time per instrument can be run, which is not optimal for high-volume work. Open systems require more hands-on time with longer time on the instrument, but they are able to run many samples at once. Unfortunately, the cost of PCR testing may be prohibitive for some laboratories.

**Comparison of Methods**
Information regarding the use of rapid methods of detection vs. stool cultures is somewhat contradictory. Several studies state that stool cultures are not as sensitive as the rapid methods for detection of *Campylobacter*. However, some rapid tests are limited to only *C. jejuni* and *C. coli*, but other species, such as *Campylobacter upsaliensis*, may cause disease in humans. These other species could be isolated in culture. Another disadvantage of rapid and molecular testing is that the organism is not growing in culture and therefore is not readily available for susceptibility testing.

**Summary**
Illness due to *Campylobacter* is a major health concern, and it is important to utilize testing that will yield timely and accurate results. The three methods listed above have advantages and disadvantages. While culturing is difficult it is highly specific. When cultured, the organism is available for antimicrobial susceptibility testing. Antigen testing is rapid and easy to do but may not be as sensitive as other methods. A few systems using PCR methodologies are available. When evaluating different PCR systems, laboratories may need to consider the trade-offs between rapid turnaround, hands-on time, and sample throughput, as these factors vary between systems. The cost of PCR testing is also a consideration.

**References**

2015 Food Safety Report. CDC Website.
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