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**Learning Outcomes**

Upon completion of this exercise, the participant will be able to:

- Discuss the clinical significance of *Campylobacter* species.
- Explain how to isolate *Campylobacter* spp. from feces and blood.
- Explain how to presumptively identify *Campylobacter* spp. isolated from stool cultures.

The taxonomy of the genus *Campylobacter* has been revised and expanded in recent years and now includes at least 14 human pathogens. These organisms cause gastroenteritis, septicemia, and periodontal disease; and they have also been implicated in abscesses, meningitis, abortions, and deep tissue infections of the head, neck, and viscera. The presence of *Campylobacter* in the bloodstream can indicate an underlying malignancy. However, of the diseases caused by campylobacters, gastroenteritis is by far the most common. In fact, surveys have shown that *Campylobacter jejuni* is the world’s leading cause of bacterial gastroenteritis.

Most cases of *Campylobacter* gastroenteritis resolve in about a week, but serious complications sometimes occur. According to the Centers for Disease Control and Prevention (CDC), *Campylobacter* causes about 124 fatalities annually in the United States. It can lead to sepsis in immunocompromised patients, and cases of meningitis, endocarditis, and reactive arthritis have also been documented. Another rare but significant complication from infections caused by *C. jejuni* is Guillain-Barré syndrome. Studies have shown that 20%-40% of persons with this syndrome are infected with *C. jejuni* 1-3 weeks before symptoms begin.

The species most often associated with gastroenteritis, *C. jejuni*, *C. coli*, and *C. lari*, are usually acquired from contaminated food (particularly poultry), milk, or water; or from contact with infected animals (especially cats, dogs, and birds). There have also been reports of person-to-person and sexual transmission. The populations most at risk for *Campylobacter* infections are children younger than 1 year of age and adults 20-29 years of age.

Most people with diarrheal illness caused by *Campylobacter* do not need antibiotics. For those who do require treatment, erythromycin is the drug of choice. Gentamicin is preferred for systemic infections, but tetracycline, erythromycin, and chloramphenicol may also be used. However, antibiotic resistance, especially to fluoroquinolones, has been increasing since these drugs were introduced for use in animals consumed as food. In 1999, according to the CDC, 18% of *Campylobacter* infections were caused by fluoroquinolone-resistant strains. Emerging drug resistance is worrisome, not only because this makes it more difficult to treat severely ill patients, but also because susceptibility testing is not routinely performed (due to the lack of standardized testing methods), thus making resistance more difficult to detect.
Isolation of *Campylobacter*

The specimens most often submitted for isolation of *Campylobacter* are feces and blood, and no special preparation is required. However, if culture will be delayed more than 2 hours, stool specimens should be transported in either Cary-Blair transport medium or *Campylobacter* thioglycolate broth. Buffered glycerol-saline should not be used because it is toxic to campylobacters.

Isolation of campylobacters from feces requires selective media and an atmosphere of 5%-10% O\textsubscript{2} and 10% CO\textsubscript{2}. Blood-free media that contain charcoal and more selective antibiotics have been shown to provide better recovery than earlier types of selective media. For best results, 2 sets of 2 selective agars should be used. To isolate *C. jejuni* and *C. coli*, one set of selective agars should be incubated at 42º C for 72 hours. To isolate *C. fetus subsp. fetus*, *C. jejuni subsp. doylei*, *C. upsaliensis*, *C. lari*, and *C. hyointestinalis*, the other set of selective media should be incubated at 37º C for 3-7 days.

*Campylobacter* spp. may require as much as 2 weeks for growth in blood culture media. Growth can be detected by CO\textsubscript{2} monitoring, microscopic examination with acridine orange stain, or blind subcultures incubated in an atmosphere of 5%-10% O\textsubscript{2} and 10% CO\textsubscript{2}.

**Selective Media Used for Isolation of *Campylobacter* spp.**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Composition</th>
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</thead>
<tbody>
<tr>
<td>Blood-free, charcoal-based selective medium</td>
<td>Columbia base with charcoal, hemin, sodium pyruvate, and antibiotics (vancomycin, cefoperazone, and cylohexamine)</td>
</tr>
<tr>
<td>Butzler</td>
<td>Thioglycolate fluid with agar, 10% sheep blood, and antibiotics (bacitracin, novobiocin, actidone, colistin, and cefazolin)</td>
</tr>
<tr>
<td>Campy-BAP</td>
<td>Brucella agar base with 10% sheep blood and antibiotics (trimethoprim, polymyxin B, cephalothin, vancomycin, and amphotericin B)</td>
</tr>
<tr>
<td>Campy-CVA</td>
<td>Brucella agar base with 5% sheep blood and antibiotics (cefoperazone, vancomycin, and amphotericin B)</td>
</tr>
<tr>
<td>Medium V</td>
<td>Modification of Butzler with cefoperazone, rifampin, colistin, and amphotericin B</td>
</tr>
<tr>
<td>Modified charcoal cefoperazone deoxycholate agar (CCDA)</td>
<td>Charcoal-based agar with antibiotics (cefoperazone and deoxycholate)</td>
</tr>
<tr>
<td>Semi-solid motility agar</td>
<td>Mueller-Hinton broth II, agar, cefoperazone, and trimethoprim lactate</td>
</tr>
<tr>
<td>Skirrow's media</td>
<td>Peptone and soy protein agar base, lysed horse blood, and antibiotics (vancomycin, trimethoprim, and polymyxin B)</td>
</tr>
</tbody>
</table>
Identification of *Campylobacter*

Typical *Campylobacter* colonies are gray to pinkish or yellowish gray and slightly mucoid or “runny.” Suspicious colonies isolated from feces may be presumptively identified as *Campylobacter* spp. if they meet the following criteria:

- **Growth at 42°C**: *C. jejuni* spp. *jejuni* (the most common cause of bacterial gastroenteritis) and *C. coli* grow at 42°C; other colon bacteria are inhibited at this temperature.
- **Oxidase and catalase positive**: Most pathogenic *Campylobacter* species are oxidase and catalase positive.
- **Characteristic curved morphology on Gram stain**: *Campylobacter* species appear as faintly-staining gram-negative rods with a characteristic “seagull-wing” shape. However, they stain poorly with safranin, so carbolfuchsin is recommended as a counterstain. Or, if safranin is used, counterstaining should be extended to 2-3 minutes.
- **Darting motility in wet preparation**: *Campylobacter* species have a distinctive darting motility when observed in a wet preparation made from Brucella or trypticase soy broth. Distilled water or saline should not be used because these appear to inhibit motility.

*Campylobacter* species can be definitively identified by commercially available latex agglutination methods or nucleic acid probes. They can also be differentiated by testing for susceptibility to nalidixic acid and cephalothin, rapid hippurate hydrolysis, production of hydrogen sulfide in triple sugar iron agar butts, nitrate reduction, and hydrolysis of indoxyl acetate.

**Suggested Reading**