Failure of *Campylobacter pylori* To Grow in Commercial Blood Culture Systems

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Of 50 blood culture sets, 20 Bacto (Difco Laboratories, Detroit, Mich.), 20 Septi-Chek (Hoffmann-La Roche, Inc., Nutley, N.J.), and 10 BACTEC 6B and 7D (Johnston Laboratories, Inc., Towson, Md.) sets were inoculated with *Campylobacter pylori* and fresh human blood. None of the 50 blood cultures produced any detectable growth. Current commercial blood culture systems may be inadequate for the detection of *C. pylori* bacteremia.

In 1983, Warren (8) and Marshall (6) reported the presence of *Campylobacter pylori* in the stomachs of patients with active chronic gastritis. Current data suggest that this organism may have a specific pathogenic role in the development of gastritis and ulcer.

Currently, it is not known if *C. pylori* is capable of causing disease outside the gastrointestinal tract. It can be isolated from gastric biopsies (4), but attempts to culture it from other tissues have been unsuccessful so far, and it has never been recovered in blood cultures. Other species of *Campylobacter* are known to occasionally give rise to bacteremia (5, 7). The lack of such findings for *C. pylori* could indicate a less pathogenic role for this organism, but could also be due to technical problems in documenting the bacteremia.

We evaluated three commercial blood culture systems to find out if they would support the growth of *C. pylori*. In particular, we wanted to find out if the routine handling of blood cultures in a hospital-based microbiology laboratory would be sufficient to detect *C. pylori* bacteremia should it occur. We tested 20 Bacto (Difco Laboratories, Detroit, Mich.) (Tryptic soy broth-Thioll broth with sodium polyanethol sulfonate and CO2), 20 Septi-Chek (Hoffmann-La Roche, Inc., Nutley, N.J.), and 10 BACTEC 6B and 7D (Johnston Laboratories, Inc., Towson, Md.) blood culture sets.

*C. pylori* isolates from 18 different patients were used, 17 from West Virginia and one from Australia (NCTC 11638). Bacteria were grown in liquid medium containing 90% bruccella broth and 10% horse serum supplemented with 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.). The cultures were incubated at 37°C in 10% CO2 atmosphere. After 5 days, when heavy growth was visible in the tubes, the broths were serially diluted to 1:1,000 in 0.9% NaCl and 0.5 ml of broth was injected into each blood culture bottle along with 5 ml of human blood. For the smaller BACTEC bottles, 0.3 ml of bacterial inoculate and 3 ml of blood were used. The human blood was obtained from healthy volunteers who were not taking antibiotics. Blood was collected fresh each time, only minutes before each bacterial inoculation. Each volunteer was used for one set only of each blood culture system. Half of the Bacto and Septi-Chek sets and all of the BACTEC bottles were supplemented with 1% IsoVitaleX.

Each inoculated set was coded and handed over to the hospital microbiology laboratory to be processed the same way as blood cultures from current hospitalized patients. This included aeration of one of the bottles and incubation at 37°C in 6% CO2 atmosphere. Bacto and Septi-Chek bottles were visually checked for turbidity daily and routinely subcultured on blood and chocolate agars on days 1, 2, 7, and 14. All subcultures were incubated in 6% CO2 at 37°C. BACTEC bottles were incubated on a 280 rpm orbital shaker (model 3520JJ; Lab Line Instruments, Melrose, Ill.) for the first 48 h. Growth index readings were done on days 1, 2, 3, 5, 7, and 14. All through the handling, the laboratory personnel were totally blinded as to the contents of each blood culture.

It has previously been shown that fastidious campylobacters, such as "C. cinaedi," can be hard to isolate from blood cultures (2). Since *C. pylori* can be grown in bruccella broth with 10% horse serum, we thought that blood cultures had the potential for sustaining growth. We anticipated, however, that *C. pylori* would need the addition of IsoVitaleX, which was necessary for growth in our liquid medium.

Contrary to our expectations, none of the 50 blood cultures produced any growth. Colony counts were done on each individual inoculum. All 50 blood cultures were shown to have been injected with viable organisms. Results of the colony counts are summarized (Table 1).

It is possible that some factor in the blood, such as neutralizing antibodies, killed the organisms, but we find this unlikely for several reasons. We used new volunteers for each blood draw, and we do not think that every one of them would have such antibacterial activity in their blood. To make sure that our organisms were not killed by the inoculation itself, the blood of one volunteer was also used to inoculate an additional four blood culture bottles which were subsequently subcultured after 15 min. All four bottles produced growth of an expected number of viable organisms despite contact with fresh blood. We also made control cultures, using both outdated human blood and sheep blood with IsoVitaleX, but none of them sustained growth. However, when the same batch of sheep blood was used in...

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NOTES

Bacto Septi-Chek grew well in the culture media. Goodwin et al. (4) noted that campylobacters contain an inhibitory ingredient in their growth yield. We hypothesized that the additives in broth media were responsible for this inhibition. Reports by Goodwin and colleagues (3) suggested that campylobacters are sensitive to sodium metabisulfite and that this additive inhibits growth in blood culture systems. We propose this additive be removed from the broth media and replaced with an equivalent volume of sterile water.

Despite these findings, patients with Campylobacter pyloridis bacteremia have been detected (5). However, the reason for this has not been determined. In this study, we sought to determine the reason why some isolates of Campylobacter pyloridis fail to grow in blood culture systems.

We tested the following broth media: Bacto broth, Bacto broth with IsoVitaleX, Septi-Chek broth, Septi-Chek broth with IsoVitaleX, and the BACTEC broth. We performed these tests in triplicate for each isolate.

Table 1 shows the results of these tests. The number of isolates that grew well in each broth media is shown in the table. The mean and standard deviation of the CFU/ml of blood for each isolate are also shown.

Table 1. Colony counts of inoculated C. pylori

<table>
<thead>
<tr>
<th>Culture set</th>
<th>No. of isolates</th>
<th>Rate* at indicated CFU/ml of blood</th>
<th>Mean</th>
<th>SD</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1-9.9</td>
<td>10-99</td>
<td>≥100</td>
</tr>
<tr>
<td>Bacto</td>
<td>10</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Bacto +</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>IsoVitaleX</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septi-Chek</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Septi-Chek +</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>IsoVitaleX</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BACTEC +</td>
<td>10</td>
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<td>5</td>
<td>4</td>
</tr>
<tr>
<td>IsoVitaleX</td>
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</table>

* Colony counts for each isolate were divided by five for the Bacto and Septi-Chek cultures and by three for the BACTEC cultures.

We failed to support the growth of C. pylori when handled under routine microbiology laboratory conditions. Regardless of the reason for this, as discussed above, it tells us that some of the currently available blood culture systems are inadequate for detection of C. pylori, and it is quite possible that bacteremia with this organism would be missed should it occur.

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LITERATURE CITED