

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

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Received 1 October 1987/Accepted 22 January 1988

**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-Thiol broth with sodium polyanetholsulfonate and CO<sub>2</sub>), 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% brucella broth and 10% horse serum supplemented with 1% IsoVitalX (BBL Microbiology Systems, Cockeysville, Md.). The cultures were incubated at 37°C in 10% CO<sub>2</sub> 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% IsoVitalX.

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% CO<sub>2</sub> 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% CO<sub>2</sub> 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 brucella broths 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 IsoVitalX, 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 IsoVitalX, but none of them sustained growth. However, when the same batch of sheep blood was used in

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TABLE 1. Colony counts of inoculated *C. pylori*

Culture set	No. of isolates	Rate <sup>a</sup> at indicated CFU/ml of blood			Mean	SD
		1-9.9	10-99	≥100		
Bacto	10	2	4	4	196	301.6
Bacto + IsoVitaleX	10	0	0	10	258	80.6
Septi-Chek	10	2	5	3	148	287.4
Septi-Chek + IsoVitaleX	10	0	0	10	258	80.6
BACTEC + IsoVitaleX	10	1	5	4	96	62.3

<sup>a</sup> Colony counts for each isolate were divided by five for the Bacto and Septi-Chek cultures and by three for the BACTEC cultures.

making solid medium for our routine isolations, *C. pylori* grew well on it.

Another possible explanation for the lack of growth is an inhibitory ingredient in the blood culture medium itself. One such factor is sodium metabisulfite which has been shown by Goodwin et al. to inhibit growth of *C. pylori* (3). However, according to the manufacturers, none of the three blood culture systems contain this additive. We are unaware of any reports that sodium polyanetholsulfonate is inhibitory for campylobacters. We cannot rule out, however, that there are other, so far unrecognized inhibitory ingredients in the blood culture media.

Despite speculation about possible inhibitory factors, we think the most likely explanation is instead absence of some vital growth factor in the medium. A blood culture medium with a broth closer to brucella broth might have been more successful. Other additives such as 1% soluble starch, as described by Buck and Smith (1), or 0.25% yeast extract, as proposed by Goodwin et al. (3), might also have increased the yield.

In summary, though, we think the most important conclusion that can be drawn from our experiment is that the Bacto, Septi-Chek, and BACTEC blood culture systems all

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.

This work was supported in part by Public Health Service grant RR05870 from the National Institutes of Health.

We are grateful to the personnel in the microbiology laboratory at St. Mary's Hospital, Huntington, W. Va., for help with incubation of the BACTEC bottles. The BACTEC bottles were donated by Johnston Laboratories, Inc., Towson, Md.

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