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Detection of *Mycoplasma hominis* Septicemia by Radiometric Blood Culture

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The ease with which *Mycoplasma hominis* can be recovered and the frequency of its occurrence in septicemia may not be fully appreciated. We detected the growth of *M. hominis* radiometrically with an automated blood culture instrument. The organism grew in both aerobic and anaerobic culture media, but the cultures were not visibly positive. It was necessary to stain the cultures with acridine orange to visualize *M. hominis* and to subculture them on Columbia base sheep blood agar to confirm the positive growth index indicated by the instrument. Sodium polyanetholesulfonate inhibited the growth of *M. hominis* and is not recommended for use as the anticoagulant when blood is cultured for *Mycoplasma* spp.

Although *Mycoplasma hominis* is normal flora of the genitourinary tract (10), the organism is also an opportunistic pathogen. It has been implicated as a cause of postpartum fever (8, 14), salpingitis (7), and pyelonephritis (11) and has been associated with obstruction, manipulation, and surgery of the genitourinary tract (4, 9, 13). It has also been isolated from patients who have suffered multiple trauma (12). The pathogenic potential of *M. hominis* in these patients was substantiated by blood culture isolation (1, 4–6, 9, 12–14). We isolated this organism from the blood of six patients with genital or pelvic disease. Positive cultures were detected by the BACTEC blood culture instrument in the absence of visible evidence of growth.

MATERIALS AND METHODS

Collection and culture of blood specimens. Blood specimens were collected in sterile Vacutainer tubes (100 by 16 mm) containing 1.7 ml of 0.35% sodium polyanetholesulfonate (SPS) in 0.85% sodium chloride (Becton Dickinson Vacutainer Systems, Rutherford, N.J.). The specimens were mixed thoroughly with the anticoagulant and transported to the laboratory. Blood was dispensed (in approximately 3-ml portions) through a sterile double-ended needle into BACTEC 6B aerobic and 7D anaerobic bottles (Johnston Laboratories, Inc., Towson, Md.). Both media contained tryptic soy broth (2.75, wt/vol), hemin (0.0005% wt/vol), vitamin K (0.00005%, wt/vol), and 2.0 μCi of ^{14}C-labeled substrates. The aerobic medium also contained pyridoxal hydrochloride (0.01%, wt/vol), sucrose (0.25%, wt/vol), and sodium bicarbonate (0.0375%, wt/vol) in a 10% CO_2 atmosphere. The anaerobic medium also contained yeast extract (0.42%, wt/vol), animal tissue digest (0.051%, wt/vol), sodium citrate (0.021%, wt/vol), sodium carbonate (0.011% wt/vol), thiols broth (0.123%, wt/vol), glucose (0.25%, wt/vol), and sodium hydroxide (0.0375%, wt/vol) in a 10% CO_2–90% N_2 atmosphere. Neither the aerobic nor the anaerobic bottle contained SPS.

The aerobic bottles were incubated at 35°C with agitation on a BACTEC shaker for 48 h and then without agitation for up to 7 days. The anaerobic bottles were incubated without agitation. Bottles were tested after 1, 2, 3, 5, and 7 days with BACTEC 460 instrument (Johnston Laboratories). A growth index (GI) of ≥30 was considered positive for the aerobic bottles and a GI of ≥20 was considered positive for the anaerobic bottles. Cultures that were radiometrically positive were Gram stained and then stained with acridine orange when no organisms were seen. Subcultures from both aerobic and anaerobic bottles were made on chocolate and Columbia sheep blood agars (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 35°C in 5% CO_2. The anaerobic bottles were also subcultured on Schaedler blood agar supplemented with hemin and vitamin K and incubated anaerobically in a GasPak (BBL) jar. All plates were examined daily for 3 days.

SPS inhibition. We placed 20 μl of 5% SPS (Roche Diagnostics, Div. Hoffmann-La Roche, Inc., N.J.) onto sterile blank disks (Difco Laboratories, Detroit, Mich.) which were then air dried. Colonies of *M. hominis* were heavily streaked onto Columbia blood agar plates, and duplicate SPS disks were placed on the inoculum. After 48 h of incubation at 37°C in 5% CO_2 the plates were examined for growth inhibition by SPS and the diameters of the inhibition zones were measured.

Acridine orange staining. Slides for staining with acridine orange were air dried and then fixed in absolute methanol for 2 min. The methanol was drained off, and the slides were flooded for 2 min with acridine orange stain (Becton Dickinson). The slides were rinsed with water, air dried, and examined for fluorescence under oil immersion at ×1,000 magnification.

Identification. Isolates were confirmed as *M. hominis* by the species-specific antiserum growth inhibition test (3) after initial identification by culture and staining characteristics. Identification was confirmed by the laboratory of M. F. Barile (Bureau of Biologics, Division of Biological Products, Food and Drug Administration, Bethesda, Md.).

RESULTS

Case histories. *M. hominis* was isolated from the blood of five female patients and one male patient during a 7-month period. Four of the women (patients 1 through 4) had postpartum fever and symptoms consistent with endometri-

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TABLE 1. GI readings of aerobic and anaerobic blood cultures containing *M. hominis*

Patient	Blood cultures		GI at day:				
	No. positive/ total	Incubation atmosphere	1	2	3	5	7
1	2/2	Aerobic	15	17	28	40[a]	34
		Anaerobic	7	8	59[a]	63	—[b]
		Aerobic	13	17	14	34[a]	32
		Anaerobic	7	7	14	48[a]	—
2	1/1	Aerobic	15	21	53[a]	10	8
		Anaerobic	5	5	35[a]	36	26
3	3/4	Aerobic	18	17	44[a]	40	33
		Anaerobic	6	52[a]	56	49	—
		Aerobic	14	17	25	38[a]	29
		Anaerobic	9	12	53[a]	52	—
		Aerobic	13	17	17	33[a]	32
		Anaerobic	6	7	9	43[a]	33
4	1/1	Aerobic	18	20	15	23	42[a]
		Anaerobic	5	5	5	6	5
5	1/2	Aerobic	19	24	21	33[a]	30
		Anaerobic	5	6	4	22[a]	36
6	2/2	Aerobic	18	19	20	16	15
		Anaerobic	1	2	2	30[a]	37
		Aerobic	17	21	21	19	19
		Anaerobic	2	4	3	4	27[a]

[a] First positive GI for culture.
[b] —, No reading at day 7.

tis. Two of these four patients (1 and 2) were admitted with preeclampsia and required cesarean section. One infant had abnormal heart tones in utero and required resuscitation at delivery. The other two women (3 and 4) had normal vaginal deliveries. Three of these four women (patients 1, 2, and 3) had onset of fever within 1 to 2 days after delivery, but patient 4 was readmitted to the hospital with fever a week after delivery. The admitting diagnosis was pyelonephritis, but routine urine cultures were negative. Results of physical examination and pelvic ultrasound appeared normal, but endometritis could not be excluded. *M. hominis* was cultured from the blood of this patient and in significant numbers from routine endometrial culture. The organism was isolated on Columbia blood agar. Endometrial cultures from patients 2 and 3 had heavy growth of *Gardnerella vaginalis* and either light or heavy growth of anaerobes. A predominance of lactobacilli and a light growth of anaerobes were cultured from the endometrium of patient 1. *M. hominis* was not isolated from routine endometrial cultures of patients 1, 2, and 3, but no special procedures are used routinely to detect this organism.

Patient 5 had a total hysterectomy and a left salpingo-oophorectomy. On the day after surgery she became febrile and complained of burning upon urination. Routine urine cultures were negative, and a vaginal culture grew large numbers of mixed aerobes and anaerobes.

The male patient (patient 6) had severe leg injuries resulting from an automobile accident. His hospital stay was complicated by genitourinary tract problems requiring a transurethral resection of the prostate. During the febrile episode in which *M. hominis* was cultured from his blood, he had oliguria and hyperkalemia. Routine urine cultures were negative.

All six patients were treated with antibiotics. Their temperatures ranged from 38.3 to 39.5°C, and in patients 4 and 5 the fever persisted for 5 days. Five patients became afebrile after treatment with gentamicin or clindamycin or both. These patients had previously received a variety of cell wall-active antibiotics, including cephalothin, ampicillin, and cefoxitin, but had remained febrile. Patient 2 became afebrile without specific treatment for *Mycoplasma* infection. In each case, all blood cultures were drawn in a single day and before clindamycin or gentamicin was administered.

Isolation from blood cultures. Table 1 shows the BACTEC GI determinations from days 1 through 7 of incubation for each of the aerobic and anaerobic bottles. *M. hominis* was recovered from both aerobic and anaerobic bottles, but in 5 of the 10 cultures, growth was detected by the BACTEC system in the anaerobic bottle only or was detected in the anaerobic bottle 1 day earlier. In only one case (patient 4) was the aerobic bottle alone positive radiometrically. The times of detection varied from 2 to 7 days, and the GI readings ranged from 22 to 63 in the anaerobic bottles and from 33 to 53 in the aerobic bottles.

Pinpoint colonies became visible on subculture after 2 to 3 days. The colonies were nonhemolytic and transparent, resembling water droplets. With continued incubation, the colony surfaces appeared rough when viewed with a stereoscopic microscope, and the colonies began to assume the more characteristic mycoplasma form. Subcultures grew best on Columbia blood agar incubated in 5% CO_2; growth was barely visible on chocolate agar incubated in 5% CO_2 or on Schaedler blood agar incubated anaerobically. Only amorphous gram-negative material was seen by Gram stain. Acridine orange stains revealed pleomorphic organisms with a predominance of coccobacillary forms. The fluorescent stain was uniform throughout the microbial cells. All bacterial isolates were identified as *M. hominis*.

Inhibition by SPS. All strains of *Mycoplasma* were inhibited by SPS. The diameters of the zones of inhibition ranged in size from 13 to 18 mm.

DISCUSSION

Female patients may have *M. hominis* as normal flora of their genitourinary tracts and therefore appear to be at risk for developing mycoplasma infection. Five of our six patients were females, and four of these had postpartum fever. Platt et al. (8) indicated that *M. hominis* was the etiological agent in 14 of 28 (50%) patients with postpartum fever. He used an increase in antibody titer of fourfold or greater as the criterion of infection. Five of seven patients with *M. hominis* septicemia reported by Boe et al. (1) had postpartum fever: three after spontaneous abortions, one after a normal cesarian section, and one had a legal abortion complicated by salpingitis. Mårdh and Westrom (7) identified *M. hominis* as the cause of salpingitis in 4 of 50 (8%) patients, and *Neisseria gonorrhoeae* was isolated in 4 (8%) of the cases. These organisms were cultured from specimens taken from fallopian tubes during laparoscopy. One of our patients became septic after a total hysterectomy and salpingo-oophorectomy. Boe et al. (1) also reported the isolation of *M. hominis* from a woman after surgery for cancer of the rectum.

In males, *Mycoplasma* infections usually follow genitourinary tract obstruction or surgery (9) or multiple trauma (12).

Our male patient was a trauma victim who had urinary tract obstruction. His urine cultures were negative, but *M. hominis* was isolated from two of his blood cultures.

Recently, the organism was reported as the cause of septicemia in three male patients: two with leukemia (4, 6) and one after major heart surgery (1). In these cases the septicemia was of long duration and required antibiotic therapy. Five of our six patients remained febrile on cell wall-active antibiotics but defervesced after being treated with clindamycin or gentamicin or both. These antibiotics have been shown to be effective against *M. hominis* (1, 12). One of our patients became afebrile without specific antimicrobial therapy. In some patients, the septicemia may be self-limited because of the immune response of the patient (6).

Investigators have stressed that special *Mycoplasma* broth media, routine subcultures, and prolonged incubation should be used if *Mycoplasma* septicemia is suspected clinically (9). Others have indicated that the organism grows in conventional blood culture broth but produces no visible growth and therefore have recommended routine subcultures incubated anaerobically or made on mycoplasma agar (12–14). Because of this reported difficulty in culturing *Mycoplasma* spp., our initial presumption was that the isolates we obtained might be cell wall-defective bacteria. However, no reversion to a parent organism occurred after repeated subculturing, and the organism never became visible by Gram strain.

Investigators differ on the capability of the BACTEC system to detect the growth of *Mycoplasma* spp. in BACTEC blood culture bottles. Evans (5) reported a case in which *M. hominis* grew in an aerobic bottle and produced a detectable GI reading. In another study (4), growth was present in aerobic and anaerobic bottles, but only one of two positive sets gave a borderline GI reading. This study indicated that routine subcultures detected the organism earlier and more consistently than did the radiometric method. Our positive cultures were detected by the BACTEC system, and both aerobic and anaerobic bottles supported organism growth. Generally, growth was detected earlier radiometrically in the anaerobic bottle. The GI readings were low but in the positive range. Of the 17 positive blood culture bottles, 2 became positive on day 7. When *M. hominis* is suspected, therefore, blood cultures may need to be incubated for a longer period of time.

Carski et al. (2) inoculated various *Mycoplasma* spp. into SPS-free BACTEC bottles and found that five of nine isolates were detected radiometrically and four by subculture only. When SPS was present in the medium, the recovery of *Mycoplasma* spp. was decreased: only two strains were detected radiometrically, five were detected by subculture only, and two isolates were not recovered. Our BACTEC bottles were supplied without SPS because the blood was drawn into Vacutainer tubes containing SPS and then transferred into culture bottles in the laboratory. The blood in the Vacutainer tubes contained 0.06% SPS. After 3 ml of blood was added to 30 ml of media in the bottles, the final SPS concentration was approximately 0.006%. When we inoculated *Mycoplasma* isolates into conventional BACTEC bottles supplied with 0.025% SPS, the number of bottles that were radiometrically positive was reduced, and fewer colonies were obtained on subculture (data not shown). In addition, all isolates had large zones of inhibition around SPS-impregnated disks. Our ability to recover *Mycoplasma* spp. from blood cultures may have been due in part to the low concentration of SPS in the blood culture medium we used, which differed from that in other commercially prepared media.

Several investigators have indicated that the growth of *Mycoplasma* spp. on agar was so slight that it was mistaken for moisture droplets or inoculum (4, 5). A Gram stain of the colonies failed to reveal organisms, which tended to confirm the absence of growth. By staining colonies with acridine orange, we were able to detect the presence of organisms on agar subcultures. Other workers also may have failed to detect the organism because subcultures were generally made on chocolate agar. In our study, the isolates grew very slightly on this medium, even with prolonged incubation. Some investigators (13) have indicated that subcultures on standard transparent mycoplasma agar facilitates the recognition of *Mycoplasma* spp. Routine clinical laboratories, however, may not be able to justify the cost, time, or personnel required for processing additional media in this way. Of the conventional media we used, we observed the best growth on Columbia blood agar and found the 5% CO_2 and anaerobic incubation atmospheres to be comparable. On the basis of these observations, we recommend that BACTEC-positive and Gram stain-negative blood cultures be stained with acridine orange, subcultured on both Columbia blood agar and chocolate agar, and then incubated in 5% CO_2 for 72 h.

M. hominis is being recognized as an important pathogen in patients with various underlying disorders and should be considered in patients with unexplained fever. Greater awareness of the ability of routine laboratory media and procedures to isolate *M. hominis*, as well as an increased knowledge of its pathogenic potential, may result in more effective isolation of this organism.

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ADDENDUM IN PROOF

Since the submission of this manuscript, we have isolated *Mycoplasma* spp. from blood cultures of five additional postpartum patients.

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