Factitious Bacterial Meningitis Revisited

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Nonviable gram-negative bacilli were seen in smears of cerebrospinal fluid from eight infants in whom bacterial meningitis was ruled out. Tubes from commercial kits were the source of the factitious organisms.

Careful examination of the Gram stain of a direct smear of cerebrospinal fluid (CSF) is critical in the immediate diagnostic evaluation of a patient with suspect central nervous system infection. A previous report by Weinstein et al. of nonviable gram-negative bacilli contaminating specimen tubes from commercial lumbar puncture (LP) trays (6) led to revision of production procedures. Nevertheless, we have again documented the presence of nonviable gram-negative bacilli in specimen tubes from LP kits. In eight pediatric patients, false-positive CSF smears were misleading, suggesting erroneous clinical diagnoses and leading to inappropriate therapy. Further laboratory studies demonstrated nonviable gram-negative organisms in tubes from adult and pediatric kits.

Laboratory and clinical observations. At the University of California Irvine Medical Center (UCIMC), a 500-bed University-County Hospital, hundreds of LPs are performed annually, using commercial LP kits. Over several months, we observed nonviable gram-negative bacilli (GNB) in sediment smears of fresh CSF from patients in UCIMC and in community hospitals. Of these patients, the eight listed in Table 1 fulfilled two criteria: (i) nonviable GNB observed on direct smears of fresh CSF sediment, and (ii) sufficient clinical information to rule out bacterial central nervous system infection. The organisms were usually sparse. The cell walls were well delineated, and the organisms were clearly distinguishable from background proteinaceous material and other debris commonly encountered in smears of clinical specimens. Most of the nonviable organisms were more suggestive morphologically of Haemophilus sp. or Pseudomonas sp. than coliform bacilli (see Fig. 1 in report by Weinstein et al. [6]).

Routine processing of CSF specimens included centrifugation, direct Gram stain of the sediment, and inoculation of chocolate agar plates and brain heart infusion and thioglycollate broths. Plates and broths were incubated (37°C in 5% CO2) for 3 to 7 days. In three patients, negative counterimmunoelectrophoresis results helped rule out meningitis due to standard pathogens (Table 1; patients 1, 2, and 4).

The clinical diagnoses and CSF findings are summarized in Table 1; in all patients, the CSF exam was of major diagnostic importance. Only the CSF specimens showing GNB are listed, but additional follow-up CSF specimens were negative. In all cases, broad-spectrum antibiotic therapy, such as with ampicillin or carbenicillin and chloramphenicol, gentamicin, or trimethoprim-sulfamethoxazole, was instituted.

Laboratory investigation of tubes from LP trays. Additional studies ruled out other sources of the apparent dead organisms, such as slides, stain, pipettes, and blotting paper. (i) CSF tubes: CSF tubes from unopened trays of Pharmaseal lot (L)C9D377 were compared with three additional Pharmaseal lot numbers and one from a different manufacturer. (ii) Sterile saline blank CSF: to each tube 6 ml of sterile filtered (Nalgene, 0.45 μm) normal saline was added, followed by centrifugation. (iii) Transfer pipettes and autoclaving: Pasteur pipettes, autoclaved either in glass jars or in paper sterilizer envelopes, were compared with sterile syringes and needles for aspiration of the sediment to prepare smears. (iv) Slides: slides from two lot numbers were rinsed with filtered (0.45 μm) 70% ethanol and compared with unrisened slides from the same lot. (v) Stains: all stains are routinely filtered (0.45 μm) daily. For this investigation, freshly filtered Harleco and Difco stains were tested. (vi) Blotting paper: air drying was compared with blotting with either bipuloser paper (p1050, Scientific Products Div., McGaw Park, Ill.) or paper towels. (vii) Cultures: all sediments from the saline-blank tubes were inoculated onto chocolate agar and into thioglycollate and brain heart infusion broths and incubated (37°C in 5% CO2) for 3 to 7 days. All cultures showed no growth.

The results revealed positive Gram stains from the blank sterile saline CSF only with tubes from samples of Pharmaseal Trays, lot numbers...
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**TABLE 1** Summary of clinical and CSF findings in patients with positive CSF smear results.

- GMS: Quantitative and sensitive.
- NA: Not applicable.
- ND: Not determined.
(L)C9D377 (all 16 tubes from four trays examined were positive for GNB) and (L)C9D144 (1 positive of 3 tubes in the single tray examined). However, none of the six tubes from trays of Pharmaseal lots (L)C9C197 and (L)C9B407 or four tubes from one lot of another manufacturer was positive. The results indicated that the polypropylene CSF specimen tubes, and not the other equipment, reagents, or procedural steps, was the source of the organisms.

Discussion. In the eight patients presented in Table 1, the organisms seen on the Gram stains did not represent true bacterial meningitis. No patients were included in whom failure of pathogenic organisms to grow was likely due to prior antimicrobial treatment. Infection with fastidious or anaerobic organisms which failed to grow in standard media plus thioglycolate or brain heart infusion broth was highly unlikely. Some CSF glucose values in patients 4, 5, and 7 were slightly lower than expected (4, 5); however, other clinical factors likely altered blood-CSF glucose transport. Because immediate, accurate microscopy of CSF is of critical diagnostic importance, misleading clinical implications and excessive antibiotic therapy resulted from the finding of factitious CSF organisms.

Conversely, another patient (not in Table 1) illustrated both the diagnostic importance of the CSF Gram stain and the danger posed by the epidemic of factitious organisms, which led to the opposite error, i.e., not reporting real organisms on a CSF smear. CSF from a 60-year-old woman with postoperative fever contained leukocytes and GNB on direct smear, but the GNB were not reported immediately because it was thought they also were factitious. However, *Proteus mirabilis* grew from this CSF. Thus, effective therapy had been delayed several hours by the assumption that the organisms in the smear were factitious.

Laboratory tests of blank CSF showed that the specimen tubes in the LP kits were the source of the organisms. Tubes from one lot had a high rate of contamination, but one additional lot was also positive. Extraneous sources, such as slides (1), stains, pipettes, or blotting paper, were ruled out. Transport media (2) or recycled glass tubes (3), other potential sources, were not used.

Because occasional nonviable organisms may be found in trays from other manufacturers (J. Morrello, personal communication), changing manufacturers may not solve the problem. Routine quality control sampling of LP kits in the clinical microbiology laboratory would not identify a low frequency of kits containing nonviable GNB and is not warranted. Rather, clinical microbiology laboratories should reinforce standard quality control procedures, such as filtration of stains and review of any specimen showing organisms on direct smear which fail to grow. The laboratory must immediately report any leukocytes, erythrocytes, and organisms seen in CSF Gram stains and also discuss the patient’s clinical status with the physician. If factitious organisms are suspect, a repeat CSF should be obtained if possible, but using new, clean sterile glass tubes (not reused [3]) instead of the tubes in the commercial kits to collect the CSF specimen.

The reemergence of factitious organisms in commercial LP kits illustrates that occasional failures of manufacturing quality control may occur (6). Clinicians and laboratories must remain alert for possible encounters with factitious organisms in CSF specimens. This problem reemphasizes the importance in the clinical microbiology laboratory of strict routine quality control of procedures, reagents, and equipment and of direct communication between the laboratory and the clinician.

LITERATURE CITED


