ORIGINAL ARTICLE

Pseudo-Outbreak of *Klebsiella oxytoca* Spontaneous Bacterial Peritonitis Attributed to Contamination of Multidose Vials of Culture Medium Supplement

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OBJECTIVE. To determine the source of a cluster of *Klebsiella oxytoca* isolates cultured from peritoneal fluid of 3 patients with cirrhosis on a single day.

DESIGN. Outbreak investigation and before-after study.

SETTING. A Veterans Affairs medical center.

METHODS. Epidemiologic investigation, analysis of antimicrobial susceptibility testing results and molecular typing of *K. oxytoca* isolates with repetitive sequence-based polymerase chain reaction (rep-PCR), review of microbiology laboratory procedures for processing peritoneal fluid cultures, and comparison of peritoneal fluid contamination rates 18 months before and after modification of laboratory procedures for culturing peritoneal fluid.

RESULTS. Each of the peritoneal fluid samples that grew *K. oxytoca* was inoculated into blood culture bottles by different clinicians at different hospital locations. None of the patients had clinical findings suggestive of peritonitis or elevated polymorphonuclear cell counts in peritoneal fluid (range, 3–25 cells/μL). Molecular typing with rep-PCR demonstrated that the *K. oxytoca* isolates were genetically related (greater than 95% similarity). Laboratory procedures included the routine addition of a culture medium supplement of yeast extract and dextrose from a multidose vial into blood culture bottles with peritoneal fluid. After discontinuing use of the culture medium supplement, there was a marked reduction in the number of peritoneal fluid cultures deemed as contaminants (14.3% vs 0.9%; $P < .001$).

CONCLUSION. A pseudo-outbreak of *K. oxytoca* peritonitis and high rates of contamination of peritoneal fluid were attributable to contamination of a multidose culture medium supplement. This article highlights the importance of discouraging the use of multidose vials in all clinical settings.

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Correct identification and early treatment of bacterial infection are crucial for the survival of patients with cirrhosis.[1] The diagnosis of spontaneous bacterial peritonitis (SBP), which results from intestinal bacterial translocation in patients with cirrhosis, is often clinically elusive and rests on the white blood cell count and microbiologic analysis of peritoneal fluid. The presence of polymorphonuclear (PMN) white blood cells has demonstrated great sensitivity in establishing SBP. Conversely, the growth of bacteria from peritoneal fluid with normal PMN counts, especially when obtained from asymptomatic patients, can be interpreted as a contaminant.[2]

Contamination of cultures may occur at any point, including processing in the microbiology laboratory. The systematic occurrence of contamination may lead to a pseudo-outbreak or a related cluster of false infections.[3] Bacterial pseudo-outbreaks attributable to contamination of cultures are often linked to the environment or equipment in the microbiology laboratory, although in some instances it has been difficult to identify the exact source for the contamination of cultures.[4-6] Here, we describe a pseudo-outbreak of spontaneous bacterial peritonitis due to *Klebsiella oxytoca* that we suspect was caused by contamination of multidose vials of a supplement added to blood culture bottles to increase the yield of peritoneal fluid cultures.

METHODS

Setting

The Louis Stokes Cleveland Veterans Affairs Medical Center is a 225-bed acute-care facility, associated with a 165-bed long-term care facility and 13 outpatient clinics, that serves

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TABLE 1. Cell Count and Differential and Gram Stain of Peritoneal Fluid for the 3 Case Patients with Cultures Positive for *Klebsiella oxytoca*

Variable	Case 1	Case 2	Case 3
Red blood cell count, cells/μL	1,000	>1,000	540
White blood cell count, cells/μL	159	162	75
Polymorphonuclear cell percentage, cells/μL (%)	3 (2)	8 (5)	22 (29)
Gram stain	No cells or organisms	No cells or organisms	No cells or organisms

more than 100,000 patients each year. Approximately 300 peritoneal fluid specimens from patients with cirrhosis or with intra-abdominal infections are analyzed every year in the microbiology laboratory. Since 2001, only 10 cultures of peritoneal fluid have demonstrated growth of *K. oxytoca*. On February 8, 2011, we were alerted by the microbiology laboratory about peritoneal fluid specimens from 3 different patients that grew gram-negative rods, eventually identified as *K. oxytoca*. Given that unusual coincidence, we initiated an outbreak investigation.

Review of Cases

SBP was defined as a peritoneal fluid analysis with a PMN cell count greater than 250 cells/μL[2], with or without bacterial growth in peritoneal fluid cultures, and no evidence of secondary peritonitis, defined as an intra-abdominal infection extending to the peritoneal space because of perforation of a hollow viscus, bowel necrosis, or associated with the presence of a peritoneal dialysis catheter.[7] Contamination of peritoneal fluid cultures was defined as growth of bacteria in peritoneal fluid culture with peritoneal fluid PMN cell count less than 250 cells/μL and the absence of documented fever, abdominal pain, nausea, or vomiting at the time of the procedure. Demographic characteristics, reason for hospital admission, and nature of underlying chronic liver disease were extracted from the medical record.

Characterization of Bacterial Isolates

The VITEK2 system (bioMérieux) was used for bacterial identification and antimicrobial susceptibility testing. Results were interpreted according to breakpoints defined by the Clinical and Laboratory Standards Institute (CLSI).[8] Molecular typing was performed using repetitive sequence-based PCR (rep-PCR) for 4 isolates from peritoneal fluid (1 each from the first 2 patients and 2 from the third patient) as well as in 2 isolates from urine cultures from unrelated patients, all obtained during the same week in February 2011. Genomic DNA was extracted from bacterial isolates using the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories). PCR amplification was performed using the DiversiLab (bioMérieux) *Klebsiella* fingerprinting kit, and rep-PCR amplicons were separated by electrophoresis on microfluidic chips and analyzed with the Agilent 2100 Bioanalyzer (Agilent Technologies). The resulting band patterns were compared by Pearson correlation, and isolates that were more than 95% similar were considered to be genetically related.[9]

Investigation of Microbiology Laboratory Procedures

To detect potential mechanisms of contamination, the location and form of collection of peritoneal fluid cultures were noted. Procedure notes were reviewed and, when necessary, the practitioner was contacted. Microbiology laboratory procedures pertaining to peritoneal fluid cultures were reviewed in detail and laboratory personnel were interviewed. To assess the possibility of an environmental source, we cultured medium supplements used in the processing of peritoneal fluid cultures.

Frequency of Peritoneal Fluid Contamination before and after Discontinuation of the Multidose Culture Medium Supplement

We reviewed the microbiology laboratory records to determine the frequency of occurrence of *K. oxytoca* in peritoneal fluid. We compared the results of peritoneal fluid cultures coupled with cell count analysis in the 18-month periods before and after discontinuation of the multidose culture medium supplement. The proportion of positive peritoneal cultures, discriminated between SBP, secondary peritonitis, and contamination (as defined above), was noted. Information collected on each specimen included hospital location, date and time of collection, result of peritoneal fluid culture, peritoneal fluid white blood cell count, associated symptoms, intra-abdominal infection, and underlying liver disease.

Statistical Analysis

Fisher exact test was used to compare proportions of categorical variables. Analyses were performed using R, version 2.15.2. *P* values less than .05 were considered significant.

RESULTS

Review of Cases

In all 3 cases, peritoneal fluid was obtained from patients with underlying cirrhosis and ascites. SBP was considered unlikely on the basis of their clinical features and peritoneal fluid parameters (Table 1).

Patient 1, a 61-year-old man, was admitted to the hospital because of maroon stools and coffee ground emesis. He did not have fever or abdominal pain. He had hepatitis C virus infection and a history of alcohol abuse, complicated by cirrhosis, portal hypertension, and ascites. On presentation, he was afebrile and hemodynamically stable. He was anemic and

had leukocytosis. He underwent paracentesis on the day of the admission in the intensive care unit (February 7, 2011). He was found to have bleeding due to esophageal varices, which were banded. The patient died a month later after recurrence of bleeding.

Patient 2, a 57-year-old man with a history of alcohol abuse, cirrhosis, and portal hypertension required paracentesis approximately every 2 weeks to alleviate ascites refractory to diuretics. He underwent paracentesis on February 7, 2011, as an outpatient in the gastroenterology procedure suite and returned home. When he was contacted to discuss the results of peritoneal fluid cultures, he denied any fever, abdominal pain, or other symptoms.

Patient 3, a 42-year-old man, was admitted to the hospital because of worsening ascites in the setting of alcohol abuse, cirrhosis, and chronic portal vein thrombosis. He had previously experienced alcohol withdrawal and delirium tremens and was actively drinking until 5 days before admission. He had leucocytosis and elevation of transaminase levels but was afebrile. He had urine and blood cultures that showed no growth. To rule out spontaneous bacterial peritonitis, he underwent paracentesis on February 7, 2011, while hospitalized on a medical ward. He was initially treated with ceftriaxone, but it was discontinued after PMN cell count was found to be inconsistent with SBP.

In all cases, peritoneal fluid was directly inoculated into blood culture bottles at the bedside immediately after paracentesis to increase the diagnostic yield of cultures,[10] and in each case, a different practitioner was involved. Therefore, it was deemed unlikely that contamination with *K. oxytoca* occurred during specimen collection. Furthermore, the patients were hospitalized in different wards, and no overlapping stays in the same inpatient wards, emergency room, imaging facilities, and outpatient clinics occurred in the previous month.

Investigation of Microbiology Laboratory Procedures

Upon review of procedures for culture processing, we learned that, in all cases, the same microbiology laboratory technician added BD Difco Supplement B (Becton Dickinson) to blood culture bottles inoculated with peritoneal fluid. The supplement contains hematin, yeast extract, L-glutamine, and dextrose, and its purpose is to increase the yield for fastidious organisms, such as *Hemophilus influenzae* and *Neisseria gonorrhoeae*. The supplement was in a 10-mL multidose vial kept in the refrigerator at 3°C between doses. According to the laboratory technician, a sterile syringe and needle were used to aspirate each dose of the supplement from the multidose vial after cleaning the cap with an alcohol pad. The same supplement bottle was used to inoculate each of the positive peritoneal fluid cultures. Unfortunately, by the time the outbreak was noted, the vial had been discarded and was not available for culture. Cultures of 3 unopened supplement bottles from the same lot showed no growth.

Characterization of Bacterial Isolates

All isolates had the same antimicrobial susceptibility profile; they were resistant to ampicillin but susceptible to gentamicin, cephalosporins, β-lactam/β-lactamase inhibitor combinations, ciprofloxacin, and trimethoprim-sulfamethoxazole. Molecular typing with rep-PCR revealed that *K. oxytoca* isolates from peritoneal fluid from all 3 patients shared more than 95% similarity among them and with 1 of the 2 contemporary urine isolates (Figure 1).

Frequency of Peritoneal Fluid Contamination before and after Discontinuation of the Multidose Culture Medium Supplement

In the 18-month period before the discovery of the pseudo-outbreak, 328 peritoneal fluid samples from 146 unique patients were cultured, and bacteria grew in 58 specimens from 35 different patients; *Staphylococcus* species, often considered a skin contaminant, was cultured from 33 specimens. In the 18-month period after discovery of the pseudo-outbreak and discontinuation of the use of the multidose culture medium supplement, 425 peritoneal fluid specimens from 170 patients were cultured, and bacteria grew in only 18 specimens; *Staphylococcus* species was cultured from 1 specimen. Figure 2 shows the percentage of positive cultures in 6-month intervals before versus after discontinuation of the multidose supplement, separated into those deemed to represent SBP, secondary peritonitis, and contaminants. The percentage of contaminants decreased from 47 (14%) of 328 to only 4 (0.9%) of 425 after discontinuation of the supplement ($P < .001$).

DISCUSSION

We describe a pseudo-outbreak of *K. oxytoca* SBP involving 3 patients with advanced liver disease and cirrhosis. Our find-

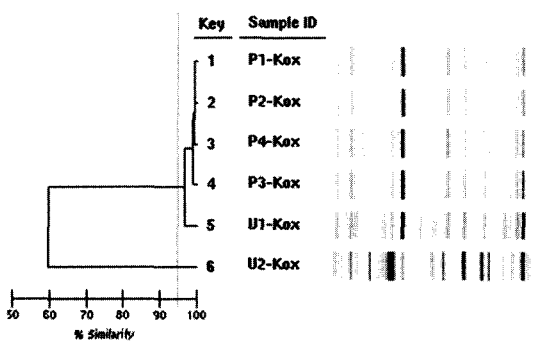

FIGURE 1. Results of molecular typing with repetitive sequence-based polymerase chain reaction. P1-Kox and P2-Kox are *Klebsiella oxytoca* isolated from peritoneal fluid obtained from patients 1 and 2, respectively. P3-Kox and P4-Kox were isolated from patient 3. U1-Kox and U2-Kox are *K. oxytoca* isolated from contemporary urine samples of unrelated patients. Based on Pearson correlation, *K. oxytoca* from all 3 patients are more than 99% similar and more than 95% similar to U1-Kox.

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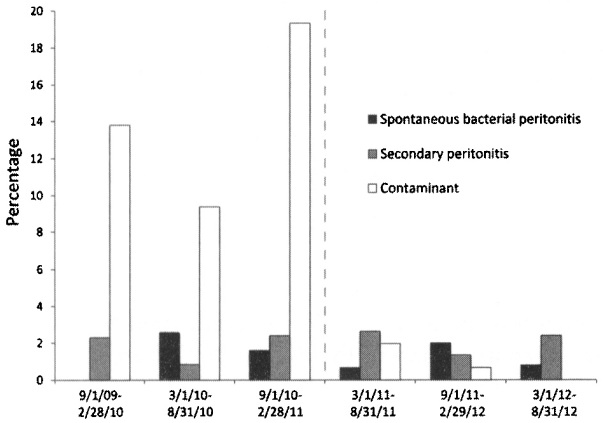

FIGURE 2. Percentage of peritoneal fluid cultures that grew bacteria, classified as spontaneous bacterial peritonitis, secondary peritonitis, and contamination, from September 2009 through September 2012. The pseudo-outbreak of *Klebsiella oxytoca* was detected in February 2011, and the use of the culture medium supplement for fastidious organisms was discontinued in March 2011, signified by the interrupted line.

ings suggest that none of the patients were truly infected, nor were they linked to a common hospital location or practitioner. Rather, their peritoneal fluid samples were inoculated in the microbiology laboratory with culture medium supplement from a multidose vial. All samples grew *K. oxytoca* isolates that were determined to be genetically similar by rep-PCR. We postulate that the multidose vial of culture medium supplements may have been accessed by a needle or syringe that was previously used on a sample from an infected patient or that was inadvertently in contact with contaminated equipment or surfaces. After introduction of bacteria into the medium supplement, subsequent contamination of the 3 cultures with the same organism may have occurred. Unfortunately, we were unable to culture *K. oxytoca* from the vial, because it had been discarded.

Contamination of multidose vials has been linked to numerous true outbreaks of infection with viral and bacterial pathogens.[11-13] Although multidose vials may be more efficiently stored and reduce waste and cost, the Centers for Disease Control and Prevention recommends against their use, given the high risk of contamination and transmission of infection.[14] In the microbiology laboratory, contamination of multidose vials of culture medium supplement has led to a pseudo-outbreak of bloodstream infections involving *Mycobacterium chelonae* in patients with AIDS.[15] Patients with cirrhosis who frequently undergo investigation for SBP may represent a group at particular risk for contamination of cultures if supplements are used, as illustrated by this article and by a pseudo-outbreak of *Bordetella bronchoseptica* linked to the use of rabbit blood to supplement culture media.[16]

We suspect that the use of culture medium supplement in our microbiology laboratory contributed to multiple previous instances of contamination of peritoneal fluid cultures. After the practice of supplementing cultures to enhance growth of fastidious organisms was abandoned, contamination decreased dramatically, whereas the proportion of true cases of SBP remained unchanged (Figure 2). Although culture medium supplements may enhance the growth of *N. gonorrhea* and *H. influenzae* from cultures of other sterile body fluids, supplementation does not appear to increase the diagnostic yield of peritoneal fluid cultures.[17] On the other hand, increased rates of contamination have been observed with the use of medium supplements. For instance, other investigators found a high proportion of contaminated cerebrospinal fluid cultures supplemented with Difco Supplement B (12%), similar to what we observed.[18]

In summary, our report demonstrates the value of periodically reviewing contamination rates and handling practices for different types of specimens to detect pseudo-outbreaks and other systematic errors in the microbiology laboratory. We suggest avoiding unwarranted routine microbiological analyses and unnecessary supplementation of clinical samples as a strategy to prevent contamination, false-positive results, and inappropriate administration of antimicrobials. Finally, our report emphasizes the importance of eliminating or reducing as much as possible the use of multidose vials in every clinical setting, including the microbiology laboratory.

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