A 45-year-old alcoholic man, not on regular follow-up with the hospital, was admitted in October 2005 with drowsiness, altered sensorium and inability to answer questions appropriately over the past 48 h. On examination, he was found to be pale, dehydrated, febrile, icteric, confused and disoriented, with bilateral pedal oedema, and was haemodynamically stable. Examination of the respiratory system revealed decreased air entry at the bases with diffuse ronchi. Abdominal examination revealed free fluid with oedema of the abdominal wall.

A complete blood count, urine examination, chest radiograph as well as an abdominal ultrasound was performed. The free fluid (ascitic fluid) was aspirated under ultrasound guidance as he was febrile and sent for clinical pathology analysis and cultures. Two sets of blood cultures (each set consisting of an aerobic, an anaerobic and a fungal culture) were drawn over a 24 h period and the patient was administered piperacillin-tazobactam 4.5 gm eight hourly and ciprofloxacin intravenous (IV) 200 mg twice a day in addition to other supportive therapy, such as IV albumin supplementation and transfusion of blood products. The antibiotic regimen was changed to meropenem IV 1gm thrice a day on the fourth day of admission based on lack of clinical improvement (persistent fever and no significant decrease in the ascitic fluid cell count). However, he developed features of acute tubular necrosis, progressed to metabolic acidosis, became hypotensive and was mechanically ventilated. He became comatose and died of sepsis and multiorgan failure 6 days after admission.

The patient had a haemoglobin of 7.1 gm%, total white blood cell (WBC) count of 30,600 cells/mm³, with neutrophilic leucocytosis and a shift to the left with toxic granulations and marked thrombocytopenia (platelet count 40,000/mm³). The ascitic fluid was yellow, hazy and showed a WBC count of 10,000 cells/mm³ with a differential count of 95% neutrophils and 5% lymphocytes. The gram-stained smear showed few gram-negative bacilli set against a background of degenerated polymorphonuclear leucocytes.

Around 5 mL of the ascitic fluid was inoculated into an aerobic and anaerobic blood culture vial each (Becton Dickinson, Cockyesville, MD, USA) and the rest of the fluid was centrifuged at 1500 rpm for 10 min. The sediment was plated onto blood agar (5% sheep blood), chocolate agar, MacConkey agar, brain heart infusion agar and Sabouraud's dextrose agar. Additionally, the deposit was also inoculated onto blood agar with 2% Tween 80, which was prepared according the procedure described in the literature. Four smears were prepared for a gram stain, methylene blue stain, periodic acid–Schiff stain and Ziehl–Neelsen stain for acid fast bacilli. The remaining portion of the fluid was treated with two to three drops of TritonX-100 (BDH Poole, Dorset, UK) for 30 min and then centrifuged at 3000 rpm for 10 min. The deposit so obtained was inoculated onto a second set of agar plates as mentioned before, except the Tween 80 blood agar plate. This procedure has been described in the literature. All pates were incubated at 37°C and 5–6% CO₂ for 48 h.

The plates inoculated with the untreated deposit failed to show any growth after 48 h of aerobic incubation. The Bactec vials inoculated with the ascitic fluid did not show any growth. The plates containing the Triton X treated material as well as the Tween 80-incorporated blood agar showed a growth of Klebsiella pneumoniae [Figures 1 and 2]. A sparse growth of coagulase-negative staphylococci was also obtained on one of the plates and this was considered as a contaminant as the same was not seen on the smear. The blood cultures accompanying the ascitic fluid also showed a growth of K. pneumoniae. The isolates were susceptible to all third- and fourth-generation cephalosporins, aminoglycosides (gentamicin, amikacin, netilmicin and tobramycin), quinolones (ciprofloxacin, ofloxacin, moxifloxacin and gatifloxacin),
β-lactam–β-lactamase inhibitors (ticarcillin–clavulanic acid, piperacillin-tazobactam and cefoperazone–sulbactam) as well as the carbapenems (imipenem, meropenem and ertapenem) using the British Society for Antimicrobial Chemotherapy methodology for antimicrobial susceptibility testing.[4]

Discussion

This was an alcoholic adult with cirrhosis of the liver, portal hypertension complicated by primary peritonitis and bacteraemia. It is common for ascitic fluid cultures to be sterile, even when the leucocyte counts are more than 500 cells/mm³.[5] This entity is called CNNA.

The use of Tween 80 and Triton-X has been previously reported in the processing of CAPD fluid.[2,3] Both the aforementioned agents are 100% pure, self-sterilising, non-anionic surfactant agents. They yield a better growth of organisms as compared with direct plating methods. This could be achieved either by liberating intracellular bacteria before they are killed and/or separation of bacteria by a reduction in surface tension, thus allowing growth of more colony forming units on the primary and secondary inocula. The same holds true for the Tween 80-incorporated blood agar. This plate in addition allows for a more ready appreciation of contaminants and mixed cultures than methods based on enrichment broth as well as an earlier growth of pathogens.[3] Earlier studies have documented the use of these agents but in CAPD fluids alone[2,3] and not for other body fluids. We report the use of these agents in processing the ascitic fluid from a case of SBP and hope that these results would pave the way for their use in other body fluids in the clinical laboratory. Direct plating techniques employed with ascitic fluid and other body fluids are frequent causes of culture-negative results and could categorise a sizeable number of our patients as suffering from CNNA.[6] CNNA, as diagnosed by standard criteria,[7] is a distinct clinical entity where cultures are sterile and, therefore, the antibiotic therapy is not guided by antimicrobial susceptibility test results. Supplementation of media (with Tween 80) or techniques (use of Triton X and inoculation of the treated specimen onto non-selective culture media) coupled with routine inoculation of blood culture vials will facilitate the isolation of organisms from body fluids (which would otherwise be reported as sterile) and help guide antimicrobial therapy in such patients.[8] A unique feature of this case is that we could obtain growth of K. pneumoniae only after using Triton X and Tween 80-incorporated blood agar as described above. While 5 mL of the fluid was inoculated into blood culture (BACTEC) vials, they did not yield a growth of organisms even at the end of the 7-day incubation at 37°C. The concentration at which Tween 80 is used is important as a higher concentration may prove toxic to bacteria. From our observations, it appears that body fluids with a rich neutrophilic infiltrate from patients with clinically suspected primary peritonitis may have sequestered organisms within neutrophils, which are available for culture only when they are released from their intracellular milieu. The cells remain aggregated and clumped together, leading to spuriously low or no viable counts on conventional media. Hence, it is important for clinical laboratories to make a concerted effort at increasing the isolation rates of organisms from body fluids using these agents. This helps in direct therapy of patients suffering from primary peritonitis.

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