Bacteraemia caused by *Clostridium symbiosum*: Case report and review of the literature

Introduction

Clostridium species are an important cause of bacteraemia, accounting for about 1% of all significant blood culture isolates. Most of these cases are caused by *C. perfringens,* but infections caused by other clostridial species are being reported with increasing frequency. These organisms may enter the bloodstream following a breakdown of the intestinal mucosal barrier. The presence of clostridial bacteraemia is often associated with certain underlying diseases including neoplasms (particularly colon cancer), immunodeficiencies, chronic renal insufficiency, decubitus ulcers, perforation of the viscus and appendicitis and advanced age.[1] This report describes a case of *C. symbiosum* bacteraemia in a woman with progressive metastatic ovarian cancer.

Case Report

A 62-year-old woman, diagnosed with stage 3C mucinous ovarian adenocarcinoma who had her first debulking surgery in November 2009, was referred to the gynaecology clinic of a University Hospital because of urinary retention. Laparotomy revealed a 25-cm semisolid pelvic mass and a large malignant-appearing tumour metastasis to the bladder, liver, rectosigmoid, appendix, omentum and intra-abdominal walls. She underwent bilateral salpingo-oopherectomy, total hysterectomy**,** omentectomy, partial vesical and large bowel resection and debulking surgery. A prophylactic injection of cefazolin (2 g) was administered before surgical incision and this antimicrobial therapy was continued for 3 days. Fourteen days after surgery the patient suffered from fever (39°C) and vaginal bleeding. The materials collected from throat, wound and urine from both nephrostomy tubes was sent for culture. She received piperacillin-tazobactam (4 × 4.5 gr. i.v.) therapy empirically after a set of BacT/Alert FAN (bioMerieux Inc., Durham, N.C.) aerobic and anaerobic blood cultures were drawn. The wound culture yielded ESBL *Escherichia coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. The *P. aeruginosa* isolate was also recovered from right nephrostomy catheter urine. The intravenous piperacillin-tazobactam therapy stopped, and meropenem (2 × 1 gr/day, i.v.) and ciprofloxacin (2 × 400 mg/day, i.v.) were started.

After a 24-h incubation period, the anaerobic bottle of blood culture was positive, and Gram stain of prepared slides showed Gram-negative rods. The organism failed to grow on initial subculture on solid agar media (Columbia blood agar, bio Mérieux-France) under aerobic and anaerobic (Anaerogen®, Oxoid, Hants, UK) conditions. However; a second subculture from an anaerobic bottle in an anaerobic cabin (Bactron-I, SHELLAB) resulted in dusty growth on blood agar media after 72 h of incubation, and very small grey, flat or low convex, circular colonies were obtained when the incubation period was extended to 5 days. Although the organism repeatedly stained as Gram negative, it was sensitive to vancomycin (5 μg) and resistant to the colistin sulphate (10 μg). These results suggested that it was a Gram-positive microorganism. Phenotypic identification tests, either traditional or semi automatic including Rapid ID 32A, API 20A (bio Mérieux-France) and MALDI-TOF MS were all unsuccessful in identifying the microorganism.

The organism was subsequently identified by partial 16S rRNA gene sequencing. Genomic DNA was extracted from bacterial cells by heating protocol. An approximately 800-bp gene was amplified by PCR using universal primers 8UA (5'-AGAGTTTGATCCTGGCTCAG-3') and 907B (5'-CCGTCAATTCMTTTAGTTT-3'), and directly sequenced with an ABI 3100 Avant Genetic System (Applied Biosystems).[2] The BLAST software available at www.ncbi.nlm.nih.gov was used to search for DNA nucleotide sequences against similar nucleotide sequences in the database.[3] A comparative search showed 99% nucleotide identity to previously registered sequences of the 16S rRNA gene *of Clostridium symbiosum* strains (GenBank accession no.M59112, EF025909 and EF442669) and helped confirm the species identification of the present isolate.

The bacterium did not produce beta-lactamase when tested with a nitrocefin disc (bioMe´rieux). Antibiotic susceptibilities were determined by E-test (bioMe´rieux) on Brucella agar (Oxoid) supplemented with 5% defibrinated sheep blood. The results showed sensitivity to sulbactam-ampicillin, amoxicillin-clavulanic acid, meropenem, clindamycin and metronidazole. We also detected susceptibility to ciprofloxacin (MIC: 38 mg/mL) which was tested since the patient was on antibiotic therapy with ciprofloxacin and meropenem at that time. The patient's fever disappeared 48 h after administration of empirical piperacillin-tazobactam therapy. The clinical condition improved considerably and at day 11 the patient was discharged with oral ciprofloxacin (1 g daily) for an additional 3 weeks.

Discussion

We reviewed the English-language literature by means of MEDLINE and found only two cases of human bacteraemia caused by *C. symbiosum.* Elsayed

and Zhang,[4] reported the first case of human infection caused by *C. symbiosum* in a 70-year-old male patient who was cachectic and severely immunocompromised with metastatic cancer of the colon. Decousser *et al*.,[5] reported the second case, a previously healthy 54-year-old man who had no history of malignancy or underlying hepatobiliary tract disease. In the second case, the risk factor for anaerobic bacteraemia was his surgical management comprising sigmoidectomy and colorectal anastomosis for recurrent diverticular sigmoiditis. In our case, the patient with metastatic ovarian cancer had debulking surgery as well as partial sigmoid resection. Independently of the presence of immunodeficiency, the three patients presented with an underlying bowel disease that created a predisposition for a bacteraemia.

Since the patients had fever with underlying disease, *C. symbiosum* recovered in the blood culture of the previous two patients was suggested as clinically significant. Two separate blood culture sets were positive for *C. symbiosum* in the first case reported by Elsayed and Zhang,[4] but only one anaerobic vial out of two separate sets yielded a positive culture in the second case.[5] In the present case, we have concluded that the isolate was clinically significant, even though there was only one positive blood culture bottle, because of the patient's predisposition to a clostridial infection, her clinical presentation and underlying malignancy. It seems likely that clostridial bacteraemia detected with only one positive blood culture does not contradict the clinical significance of the isolates. Whoo *et al*.,[6] recovered *Clostridium* sp. from single blood culture of 85% of patients associated with clinically relevant clostridial bacteraemia (n: 38).

This organism was previously included within Gram-negative genera, known as *Fusobacterium symbiosum* due to its Gram-negative staining characteristics, but it was later reassigned to the genus *Clostridium* by using more advanced techniques, such as 16S rRNA gene sequencing. Phenotypically, *C. symbiosum* is a non-toxin-producing strict anaerobe, and motile. Spores are rarely seen, but when present, they are oval and subterminal.[7] Although a number of biochemical tests can be used to differentiate this organism from other *Clostridium* spp., these traditional phenotypic methods have limited value in identifying slow-growing anaerobic bacteria. Molecular diagnostic methods such as 16S rRNA gene sequencing have been shown to be helpful in the identification of these bacteria.[8] Partial 16S rRNA gene sequencing of our patient's blood culture isolates resulted in definitive species identification.

Many laboratories do not identify anaerobes, especially to the species level. However, identifying some of the clinically common clostridial isolates is necessary, since susceptibility is variable and unpredictable.[9,10] It is important, therefore, to perform susceptibility testing to occasionally recovered isolates to produce information on their susceptibility profiles. The susceptibility test of the isolate results was consistent with the empirical treatment and the clinical condition improved considerably.

In conclusion, due to the high mortality rate associated with clostridial bacteraemia, prompt diagnosis and proper management play a major role in preventing mortality and morbidity. We suggested that this case contributes to the literature on *C. symbiosum* which is an uncommon cause of bacteraemia in patients with certain underlying diseases.

Nucleotide sequence accession number. The sequence for the *C. symbiosum* isolate from this study was submitted to GenBank under accession number JX089965.

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