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A case of bone marrow infection by *Staphylococcus saccharolyticus*

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Abstract. – We report a case of fatal bone marrow infection caused by *Staphylococcus saccharolyticus* in a 26 year old female. The causative organism was isolated by anaerobic culture on blood agar, and was identified by PCR amplification of the gap gene and genotyping of the resultant sequence.

Key Words:
Staphylococcus, Bone marrow, *Gap* gene, PCR, Genotyping.

Case Report

In August 2012, a previously healthy 26-year-old female was admitted to the clinic with fever. About three months earlier (May 15th, 2012), a high fever of 39.0°C had onset after the patient complained of headache. Symptomatic treatment and traditional Chinese medicine treatment in the local hospital was ineffective. Results from a blood test were as follows: white blood cell (WBC): 17.7×10^9, red blood cell (RBC): 3.35×10^{12}, hemoglobin (H_b): 98g/L, platelet (Plt): 386×10^9, which indicated bacterial infection. Rheumatology associated antibody titers such as antinuclear antibodies (ANA), anti-double-stranded DNA antibodies (α-dsDNAA_b), anti-Jo-1 antibody, anti U1 ribonucleoprotein (U1RNP) antibody, anti-β glycoprotein antibody, anticardiolipin antibody (ACA) were negative. Tuberculosis-specific cellular immune tests including TB-CHECK test, TB-DOT test, and TB-A_b test also showed negative results. Physical examination found swollen cervical lymph nodes. Puncture biopsy on lymph nodes was performed, which indicated necrotizing lymphadenitis. Bone marrow smears showed no patent abnormality. Treatment of the infection was performed in the local hospital. The patient was intravenously infused with penicillin (6.4 million units per 4 hours) and vancomycin (0.8 g twice a day). After two days of treatment, the patient showed convulsions and loss of consciousness. Symptomatic treatment, including intracranial pressure reduction, sedation, and the administration of an antiepileptic was carried out immediately. Ultrasonography displayed an enlarged spleen (4.6 cm × 14.4 cm) and a large amount of ascites. Examination on ascites from peritoneocentesis showed 32 g/L of protein and negative acid-fast bacilli. Imaging with computerized tomography (CT) showed normal head and neck morphology and increased lung markings near the lung bases. Intermittent spikes and slow waves were displayed by electroencephalography (EEG). The patient continued to suffer from persistent fever.

On August 31st, 2012, the patient was referred to our hospital. Results of physical examination were as follows: body temperature 36.0°C, pulse 92, breathing rate 18 times/min, blood pressure 108/50 mmHg, left supraclavicular lymph nodes (3.0 cm × 2.0 cm), bilateral inguinal and lymph nodes (3.0 cm × 2.0 cm on the right, 2.0 cm × 1.0 cm on the left). The lymph nodes showed clear boundaries, tough texture and no tenderness. Results of laboratory examination were as follows: WBC 12.8×10^9, RBC 2.35×10^{12}, H_b 95 g/L, Plt 59×10^9, Neutrophilic granulocytes 0.89×10^9, Blood Sedimentation Rate 106 mm/h, C-reactive

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protein (CRP) 158 mg/L, with Negative HIV antibody. Persistent fever persisted till September 1st. Both anaerobic and aerobic cultures of the bone marrow and blood were performed. On September 2nd, the patient presented a sudden chill and high fever, with a heart rate of 150 min and blood pressure of 80/50 mmHg, indicating septic shock. The symptoms did not improve after treatment with dopamine, norepinephrine and hydrocortisone sodium succinate. The patient was transferred to the ICU for further treatment on September 3rd and showed systemic petechiae (fibrinogen: 0.84 g/L, Plt: 19 × 10^9, D-dimer: 4985 ng/ml). Combination therapy with imipenem (1 g/12h) and vancomycin (500 mg/6h) was applied. Anaerobic blood culture indicated positive infection of the bone marrow while others showed negative. The positive strain was inoculated on blood agar and anaerobically cultured. On September 5th, non-hemolytic, visible white, small, flat, smooth, round colonies with a neat edge emerged on the blood agar plate. Microscopic identification showed Gram-positive cocci arranged in clusters (Figure 1). Biochemical examination was subsequently performed by an API20A kit (Biomerieux, Marcy l'Etoile France). On September 6th, the patient suddenly lost consciousness and became comatose. Arterial blood gas analysis showed metabolic acidosis with respiratory alkalosis. A sodium bicarbonate solution was immediately injected intravenously and the patient was placed on ventilator-assisted breathing. Eventually, the patient's family decided to end treatment. The patient died shortly after discharge. Post-mortem biochemical examination indicated *Staphylococcus (S.) saccharolyticus* infection (identification rate 99%, identification number 20005046). Molecular identification of the causative organism by PCR amplification of the *gap* gene was performed and the resultant amplicon was sequenced. Resulting gene sequences were submitted to GenBank for Blast alignment. Phylogenetic analysis was performed using the Neighbor-Joining method and the *gap* gene sequence from the causative organism was identified as *S. saccharolyticus* (Figure 2). Minimum inhibitory concentration (MIC) of the isolate was 1.5 μg/mL (vancomycin), 0.094 μg/mL (levofloxacin), 0.002 μg/mL (penicillin), 0.023 μg/mL (clindamycin) and 256 μg/mL (metronidazole). MIC of the quality control strain *Bacteroides fragilis* (ATCC25285) was 1.5 μg/mL for clindamycin (QC range: 0.5-2.0 μg/mL) and 0.5 μg/mL for metronidazole (QC range: 0.25-1.0 μg/mL).

In this case, leukemia was excluded by bone marrow smear and staining. Pathological analysis of the lymph node showed lymphadenitis and other base lesions were excluded. The infection was not been effectively controlled by large doses of antibiotics and continued to septic shock. However, repeated negative results from aerobic and anaerobic blood cultures failed to identify a causative pathogen. We, then, considered the possibility of rare bacterial infection. Finally, Gram positive anaerobes were observed in an anaerobic blood agar culture of the bone marrow and identified as *S. saccharolyticus*. After that, *S. saccharolyticus* was isolated from an endocarditis patient after prosthetic valve replacement[1] and a bacteremia patient with metastatic lung cancer[2]. Recently, *S. saccharolyticus* was isolated from biopsy tissues of patients with discitis and pneumonia[3,4].

S. saccharolyticus, Gram-positive cocci, is a rare type of anaerobe of the genus *Staphylococ-*

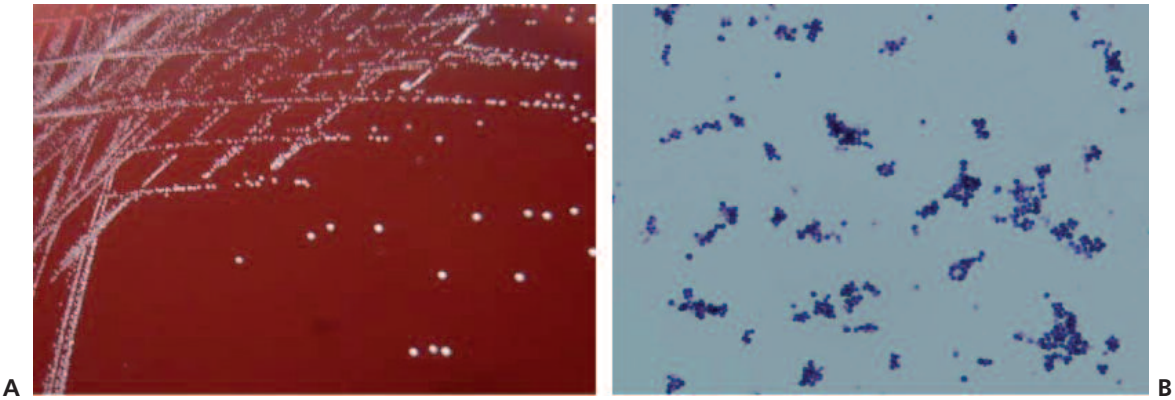

Figure 1. Colony morphology on anaerobic blood agar (**A**) and Gram stain presentation (**B**) of the clinical isolate.

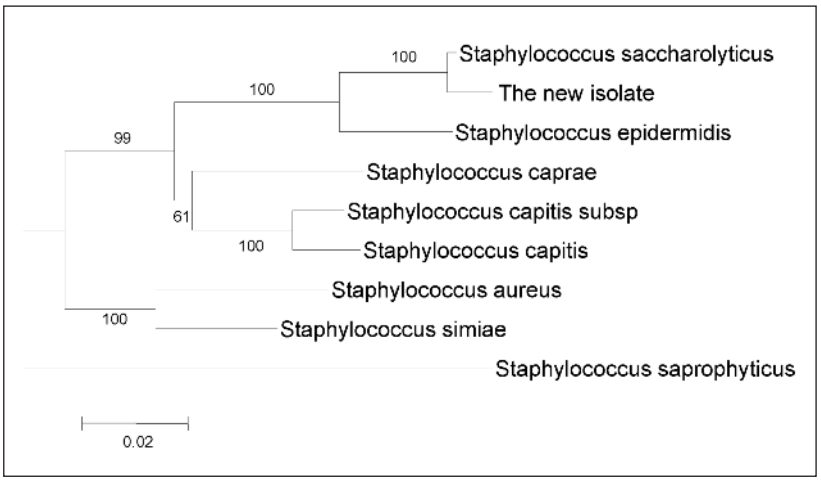

Figure 2. Neighbor-joining tree based on the 933-bp gap sequence showing the phylogenetic relationships among the staphylococcal species with the isolate from this study. The value on each branch is the percent occurrence of the branching order in bootstrapped trees.

cus, which belongs to coagulase-negative staphylococci (CNS) group. In this case, at least 7 days were needed from culture to the final identification of *S. saccharolyticus*. Godreuil et al[3] reported that 10 days were required for culture of anaerobic bacteria, while molecular methods could greatly shorten the identification time. Since the limitation of 16SrRNA in identification[5], we chose the *gap* gene, which encodes the transcription factor glyceraldehyde-3-phosphate dehydrogenase and is located in the cell wall. Upon BLAST analysis of the *gap* gene sequence obtained from the patient's isolate, only *S. saccharolyticus* showed 99% of homology.

Empirical treatment with penicillin, vancomycin and imipenem failed to control the infection in our study. Drug sensitivity tests demonstrated that *S. saccharolyticus* was sensitive to ofloxacin and clindamycin by the E-test method and sensitive to vancomycin, teicoplanin, erythromycin, pristinamycin, rifampicin and tetracycline by the paper disk method[3]. Steinbrueckner et al[2] reported that *S. saccharolyticus* was sensitive to penicillin, erythromycin, netilmicin, clindamycin, levofloxacin, and vancomycin. Wu et al[4] reported that *S. saccharolyticus* was only sensitive to levofloxacin and moxifloxacin. However, our sensitivity tests revealed that *S. saccharolyticus* was penicillin- and clindamycin-sensitive, but resistant to metronidazole. MIC of vancomycin and levofloxacin were 1.5 μg/mL and 0.094 μg/mL, respectively. *In vitro* susceptibility testing showed that the isolate was penicillin-sensitive, but a clinical therapeutic effect was not obvious, which may be related with the dose of penicillin and vancomycin. We believe that the concentration of the antibiotics in the bone marrow was not high enough, due to the bone marrow-blood barrier.

Conclusions

This is the first reported occurrence of a *S. saccharolyticus* infection of the bone marrow and we have shown that *gap* gene sequence analysis could be used in identification of *S. saccharolyticus* infections. We hope our report and review could draw attention of clinical staff on *S. saccharolyticus*.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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