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First Case of Bacteremia due to Chromosome-encoded CfxA3-β-Lactamase-Producing *Capnocytophaga sputigena* in a Pediatric Patient with Acute Erythroblastic Leukemia

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Abstract
Bacteremia due to *Capnocytophaga sputigena* occurred in a 4-year and 9-month-old Japanese girl patient with acute erythroblastic leukemia in Shinshu University Hospital, Japan. On her admission to the hospital, she had a temperature of 38.2 °C with canker sore. Prior to the commencement of chemotherapy, peripheral blood culture was carried out with the BacT/Alert 3D System ver. 4.00D (bioMérieux Japan Ltd., Tokyo, Japan) using both the PF and the SN bottles. At 48 hrs of incubation, the System showed the positive sign only in the anaerobic SN bottle for bacterial growth. The strain isolated from the SN bottle was morphologically, biochemically, and genetically characterized, and finally identified as *Capnocytophaga sputigena*. The causative *Capnocytophaga sputigena* isolate was found to be a β-lactamase-producer demonstrating to possess *cfxA3* gene. The gene responsible for the production of CfxA3-β-lactamase was proved to be chromosome-encoded, by means of southern hybridization analysis. This was the first case of bacteremia caused by chromosome-encoded CfxA3-β-lactamase-producing *Capnocytophaga sputigena*.

Key words: Bacteremia, *Capnocytophaga sputigena*, CfxA3-β-lactamase

Introduction

Capnocytophaga species are capnophilic, gram-negative fusiform rods with gliding motility, and are known to be common inhabitants in oral cavity. In immunocompromised granulocytopenic patients, a number of complications, including bacteremia, endocarditis, and peripheric lesions, have been reported to date [2, 3, 11]. An isolation of TEM-type- β-lactamase-producing *Capnocytophaga ochracea* from blood has already been reported [14]. Moreover, some isolation cases of CfxA3-type-β-lactamase-producing *Capnocytophaga* species, such as *C. granulose*, *C. ochracea*, *C. gingivalis*, together with *C. sputigena*, had been reported [6, 8]. Indeed, CfxA3-type-β-lactamase-producing strains have already been described and are well known as gene-encoded β-lactamase. However, to the best of our knowledge, no case report of bacteremia associated with CfxA3-type-β-lactamase-producing *Capnocytophaga* species has been documented. This was the first isolation of CfxA3-type-β-lactamase-producing *Capnocytophaga sputigena* from a blood stream infection.

A Clinical Case of Capnocytophaga Bacteremia

Patient: The patient was a 4-year and 9-month-old girl, transferred from other private hospital to our Shinshu University Hospital on 31 January in 2007, with the diagnosis of suspected Behçet's syndrome. She was hospitalized in a pediatric ward and finally diagnosed as erythroblastic leukemia in our hospital. On the very day of her hospitalization in our hospital, she had a temperature of 38.2 °C with canker sore, clinically suspected of having contracted bacteremia.

Isolation from the Blood of the Patient: On initiating an empirical antimicrobial dual chemotherapy with both ceftazidime (CAZ) and piperacillin (PIPC), one set of peripheral blood culture sample, that is, a PF bottle (bioMérieux Japan Ltd., Tokyo, Japan) for aerobes and an SN (bioMérieux Japan Ltd.) for anaerobes, was collected for bacteriological examination by means of the BacT/Alert 3D System ver. 4.00D (bioMérieux Japan Ltd., Tokyo, Japan).

At the 48 hrs of incubation, only the SN (bioMérieux Japan Ltd.) bottle in the BacT/Alert 3D System (bioMérieux Japan Ltd.) showed the positive signal for bacterial growth, demonstrating gram-negative, long, fusiform, straight to slightly curved rod-shaped morphology, by means of Gram stain. Hence, an aliquot (approximately 100μl) from the positive SN (bioMérieux Japan Ltd.) bottle was inoculated onto modified Drigalski (Nippon Becton Dickinson, Tokyo, Japan), Sheep Blood (Nippon Becton Dickinson, Tokyo, Japan), Chocolate agar plates (Nippon Becton Dickinson), and Anaero Columbia agar plates (Nippon Becton Dickinson), and incubated at 35 °C. After incubation for 48 hrs, yellowish colored colonies with

slightly gliding were grown both on Chocolate agar plates (Nippon Becton Dickinson) and Sheep Blood agar plates (Nippon Becton Dickinson) in 5% CO_2 incubator, and on Anaero Columbia agar plates in an anaerobic chamber. The isolate was revealed to be facultatively anaerobic Gram-negative fusiform, and capnophilic rod shaped morphology reminiscent of *Capnocytophaga* species, at a glance.

Identification of the Isolate: Biochemical characterization of the isolate was investigated with ID-Test HN20 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) kit system. Inoculated ID-Test HN20 (Nissui Pharmaceutical Co., Ltd.) kit system was kept at 35 °C in the atmosphere for 4 hrs, and final readings were carried out as stated by the instructions of the manufactures. The isolate was shown to be oxidase- and catalase- negative, and identified as *Capnocytophaga sputigena* according to the profile number 7077133 obtained. Moreover, in order to confirm the identification to the species level, 16S rRNA gene of the isolate was amplified by PCR, as previously described [15], and the resulting 1,300-nucleotides product was sequenced with an ABI PRISM 3,100 genetic analyzer (Applied Biosystems Japan, Tokyo, Japan). The sequence data obtained were compared with those of the published sequences in the GenBank database of the National Center for Biotechnology Information with the BLAST N algorithm. The closest match of the 16S rRNA sequence with *Capnocytophaga sputigena* was obtained with 99% identity (GenBank accession no. X67609), thus confirming the identification result of *C. sputigena* based on biochemical and morphological properties.

Susceptibility Tests of the Isolate: Antimicrobial susceptibility tests were carried out with the Epsilometer test (E-test, Aska Diagnostics, Inc., Tokyo, Japan) strips impregnated with gradient of different concentrations of each antimicrobial, on Mueller-Hinton agar supplemented with 5% sheep blood (Nippon Becton Dickinson) plates, as previously described [9].

The isolate was proved to be positive for the production of β-lactamase by means of Cefinase disk procedure (Nippon Becton Dickinson, Tokyo, Japan). Moreover, the isolate was demonstrated to be resistant to many β-lactam antibiotics including amoxicillin, ceftazidime (CAZ) and cefotaxime (CTX), but susceptible to imipenem, amoxicillin-clavulanic acid, CAZ-clavulanic acid, CTX-clavulanic acid and cefoperazon-sulbactam, as shown in Table 1.

Treatment was altered to intravenous administration of meropenem (500 mg, every 8 hrs. for 9 days) and clindamycin (200 mg, every 8 hrs. for 4 days). The patient's clinical state became in good condition and improved satisfactorily after intravenous administration for 7 days.

Mechanism of Resistance to β-Lactams: For the purpose of clarifying the resistance mechanism against ß-lactams, the experiment for detecting β-lactamase gene was carried out by PCR with *cfxA* -specific primers as previously described [6], and successfully found *cfxA* gene in the isolate. DNA sequencing of the PCR products was demonstrated to be *cfxA* gene. Careful analysis of the nucleotide sequence verified that the *cfxA*-β-lactamase gene coding for the isolate was different from that coding for *Bacteroides vulgatus* [12] by the substitution of two amino acids, that is, K272E and Y239D. Therefore, the β-lactamase gene of the isolate was finally confirmed to be *cfxA3*. The *cfxA3* gene was chromosome-encoded, as demonstrated by Southern hybridization study.

Table 1. Antimicrobial susceptibility of CfxA3-β-lactamase producing *Capnocytophaga sputigena* isolate as determined with Epsilometer test strips.

Antimicrobial agent	MIC (μg/ml)
Amoxicillin	>256
Amoxicillin + clavulanic acid	0.25
Aztreonam	0.19
Cefepime	3
Cefoperazone + sulbactam	0.19
Cefotaxime	>16
Cefotaxime + clavulanic acid	<0.016
Cefoxitin	0.25
Cefpirome	2
Ceftazidime	48
Ceftazidime + clavulanic acid	<0.064
Imipenem	0.064

Although several investigators reported that TEM- and CfxA-type-β-lactamases from *Capnocytophaga* species were inhibited its activities in the presence of clavulanic acid [6, 8, 14], the *C. sputigena* isolate from blood in this case was not exceptional. That is, our isolate was resistant to penicillins and cephems, but susceptible to those antimicrobials in conbination with clavulanic acid, a kind of β-lactamase inhibitor.

Discussion

The species in the genus *Capnocytophaga* are made up of slow-growing, capnophilic, fusiform, and filamentous gram-negative bacilli. They were a member of the normal gingival flora and known to cause gingivitis and periodontitis. For that reason, it has been suggested that bloodstream infections develop when *Capnocytophaga* break through the normal mucosal barrier in patients who have severe oropharyngeal mucositis or periodontal disease [2, 11].

The susceptibility of *Capnocytophaga* species to extended-spectrum cephalosporins has been reported to be variable [1, 7]. Hence, bacteremias in neutropenic patients are often empirically treated with extended-spectrum cephalosporins prior to the detection of *Capnocytophaga* species because of its slow-growing property [11]. As a matter of fact, the patient of this case was demonstrated to develope an oral ulcer probably due to the diagnosis of suspected Behçet syndrome. The site of oral ulcer might possibly be the portal of entry of *C. sputigena* in this case.

Recently, plasmid-encoded extended-spectrum TEM-17 β-lactamase, chromosome- or plasmid-encoded CfxA β-lactamase have been identified for *Capnocytophaga* species [6, 8, 15]. It is thus becoming essential to determine which antibiotics are effective against such clinical isolates. A Medline search of the literature disclosed a few clinical reports in bacteremia caused by *Capnocytophaga* species [4, 5, 10, 11, 13], however, not a single CfxA β-lactamase-producing case has been documented.

We describe here the first case of bacteremia caused by a strain of *C. sputigena* possessing a chromosome-encoded *cfxA3*-β-lactamase gene. The isolate was recovered from only an SN bottle usually inapplicable for pediatric patients. Thus indicating that this case would be difficult to be observed unless applied an SN bottle for blood culture.

In conclusion, the carbapenem or beta-lactam drug in combination of a β-lactamase inhibitor is favorable to be administered in cases of bacteremia due to CfxA3-β-lactamase producing *Capnocytophaga* species.

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