



Case report

Catheter-related bloodstream infection due to biofilm-producing *Capnocytophaga sputigena*



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ABSTRACT

Capnocytophaga sputigena is a facultatively-anaerobic bacterium that is part of the human oropharyngeal microflora. Although *C. sputigena* bacteremia is uncommon, systemic infections have been reported in both immunocompetent and immunocompromised patients. We report a case of catheter-related bloodstream infection by *C. sputigena* and highlight its enhanced biofilm-forming capacity *in vitro*.

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Introduction

Capnocytophaga sputigena is a slow-growing, capnophilic, facultatively-anaerobic gram-negative organism frequently found among the human oropharyngeal microbiota. Although typically a commensal component, *C. sputigena* has the propensity to cause infections that can manifest with oropharyngeal pathologies (e.g., periodontal disease, oropharyngeal abscesses) with the potential to extend to adjacent anatomic sites (e.g., sinusitis, keratitis, endophthalmitis, and osteomyelitis), and can even present with systemic complications such as bacteremia, endocarditis, peritonitis, and chorioamnionitis [1–3]. One case of catheter-related bloodstream infection (CRBSI) caused by *C. sputigena* has been reported [4]. Although most infections – particularly those most severe – have been described in immunocompromised hosts [1,2,5–7], immunocompetent individuals are reportedly susceptible [2]. Indeed, *Capnocytophaga* species (spp.), particularly *C.*

ochracea, have demonstrated biofilm-forming capabilities [8–11]. While biofilms notoriously confound treatment of bloodstream infections in the setting of indwelling medical devices [12–14], this pathogenic ability has not been demonstrated previously for *C. sputigena*.

Herein, we report a case of CRBSI due to *C. sputigena* infection, associated with a central venous catheter (CVC) in an immunocompromised patient. We also performed a proof-of-concept assay to highlight the ability of this clinical isolate to form biofilms *in vitro*.

Case

A 45-year old woman with poorly controlled, insulin-dependent type II diabetes mellitus, history of pulmonary embolism, and recently diagnosed systemic lupus erythematosus with lupus nephritis, presented to the emergency department with 2 weeks of abdominal distention and lower extremity swelling. In addition to insulin and blood pressure medications (amlodipine and carvedilol), she was taking apixaban, mycophenolate mofetil, prednisone, plaquenil, and furosemide at home. At admission (day 0), she was afebrile, but the exam was notable for anasarca with pitting edema to the hips and abdominal distension. Laboratory findings

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were notable for serum creatinine =6.62 mg/dL (reference 0.5–1.10 mg/dL), serum potassium = 6.6 mEq/L (reference 3.5–5.2 mEq/L), blood glucose =418 mg/dL (reference 60–100 mg/dL), hemoglobin =6.8 g/dL (reference 11.7–15.0 g/dL), total leukocyte count 6,700/ μ L (reference 4,500–11,000/ μ L), platelets = 149,000/ μ L (reference 150,000–450,000/ μ L), erythrocyte sedimentation rate =64 mm/hr (reference 0–15 mm/hr), and C-reactive protein =3.5 mg/L (reference 0.0–5.0 mg/L).

Her renal function did not improve, and on hospital day 3 she was treated with cyclophosphamide 750 mg by intravenous infusion. On hospital day 6, a double-lumen dialysis catheter was placed in her right internal jugular vein, and hemodialysis for oliguric renal failure was initiated the next day. On hospital day 13, the patient was febrile to 38.6 °C (101.4 °F) with rigors and a decreasing absolute neutrophil count = 700/ μ L (reference range 1900–8000/ μ L). Two sets of blood cultures (BD BACTEC™ FX Blood Culture System) were obtained, and empiric treatment with intravenous vancomycin and cefepime was initiated. After 48 h incubation, growth of gram-negative bacilli was noted in both aerobic blood culture bottles. Subculture to trypticase soy agar with 5 % sheep blood (blood agar), chocolate, and MacConkey agars with incubation at 36 °C in 5 % CO₂ yielded growth on blood and chocolate agars (Fig. 1A, B) approximately 24 h later. Evaluation of the growth demonstrated gram-negative bacilli (Fig. 1C), and the

isolate was identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; BD™ Bruker MALDI Biotyper™ platform) as *Capnocytophaga sputigena* with a score of 2.44.

The patient remained febrile (>38.6 °C) despite antibiotic therapy, and the internal jugular catheter was removed on hospital day 15. She was afebrile the following day and treatment with intravenous cefepime 1 g daily was continued to complete a five-day course. Repeat blood cultures (3 sets) collected on days 18–20 were without growth.

To assess biofilm formation by the *C. sputigena* isolate from this patient (C1), a microtiter plate assay for biofilm formation was performed as previously described [15]. Briefly, the clinical isolate and a *C. sputigena* reference strain (ATCC® 33612™, R1) were cultured overnight in Tryptic Soy Broth (TSB) (ThermoFisher, cat. R455052) and adjusted to a suspension with a final optical density (OD) of 0.1 (A590). Two hundred μ L of each organism suspension was aliquoted into each well of a 96-well flat-bottom microtiter tissue culture plate (Fisher Scientific, cat. 3585). To quantitate the extent of biofilm growth after 24 and 48 h in anaerobic conditions (e.g., sealed with impermeable foil at 37 °C), the OD was determined (A590) for each well prior to staining and harvest. The plates were washed twice with ddH₂O and heat fixed at 60 °C for one hour. Once cooled to room temperature, 200 μ L of 0.1 %

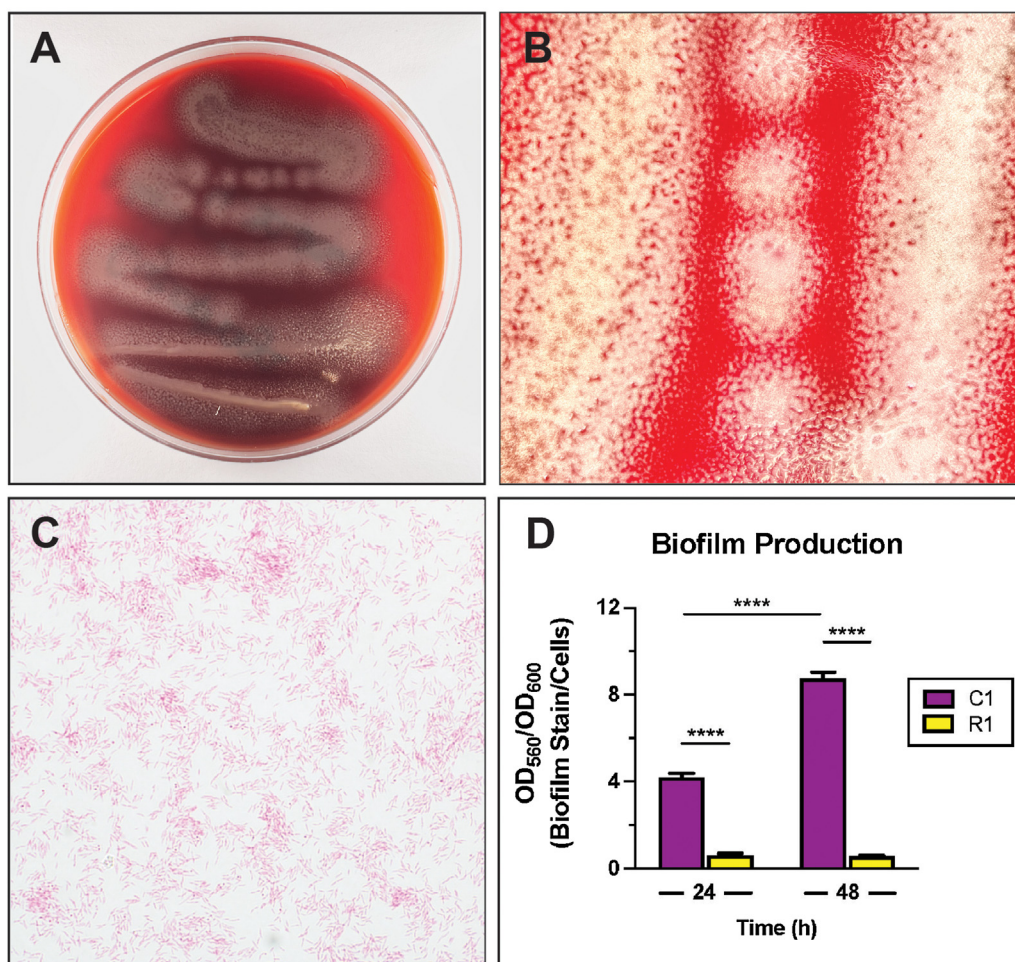


Fig. 1. Culture, Gram stain, and biofilm formation of *C. sputigena* clinical isolate.

A. *C. sputigena* isolate from patient 1 (C1) demonstrates gray-colored non-hemolytic colonies on a blood agar plate (2×) after 48 h incubation. **B.** At higher magnification (10×), swarming nature of the colonies can be appreciated. **C.** *C. sputigena* demonstrate thin, gram-negative bacilli morphology in smears prepared from the blood agar plate in A (1000×). **D.** Bar graph depicting average biofilm production quantities for the C1 and a laboratory-adapted reference strain (R1) at 24 and 48 h incubation. Error bars represent standard error of the mean and statistical differences assessed by Mann Whitney test (****, $p < 0.0001$). Note, panels A–C were generated from iPhone 12 Pro Max, Wide (1×) Lens with Moment 10× Macro Lens and Olympus DP74 microscope. Image enhancement performed with Affinity Photo, Pixelmator Pro and Topaz Labs Sharpen AI software.

crystal violet (Sigma-Aldrich, cat. 548-62-9) was added to each well for ten minutes and subsequently washed and dried overnight. Wells were de-stained with 33 % glacial acetic acid (Sigma-Aldrich, cat. A6283-1 L), and the OD was measured at A560. Biofilm formation was calculated by dividing biofilm stained OD (OD560) by OD of cells prior to staining (OD600) after adjusting to background (e.g., negative controls).

We found that biofilm production of the clinical isolate was significantly higher than that of the reference strain at both 24 h and 48 h of incubation ($p < 0.0001$) (Fig. 1D). Interestingly, unlike the reference strain, biofilm production of the clinical isolate more than doubled from 24 to 48 h incubation ($p < 0.0001$).

Discussion

The genus *Capnocytophaga*, includes a number of facultatively-anaerobic, fermenting, non-hemolytic, capnophilic, filamentous gram-negative bacilli [3,16]. To date, nine *Capnocytophaga* species are known to be part of the oropharyngeal microflora including *C. canimorsus*, *C. cynodegmi*, *C. gingivalis*, *C. granulosa*, *C. haemolytica*, *C. leadbetteri*, *C. ochracea*, and *C. genospecies AHN8471* [17,18]. These organisms can cause a variety of severe oral and systemic infections in both immunocompetent and immunocompromised hosts. Bacteremia due to *Capnocytophaga* spp. is typically reported in individuals in an immunocompromised state in the setting of active hematologic malignancy or in cytopenic patients undergoing treatment with chemotherapy [7]. Bacteremia due to *Capnocytophaga* spp. show an incidence between 1.3 and 3% of all reported cases [6,7]. Of the reported cases of *Capnocytophaga* spp. bacteremia in patients with neutropenia, *C. sputigena* makes up 22 % of the cases.

In this report, we describe catheter-associated bacteremia and isolation of biofilm-producing *Capnocytophaga sputigena* from a patient who required central venous catheters (CVC) for management of severe renal dysfunction. This represents the second case reported of CRBSI due to *C. sputigena* and the first description of biofilm formation as a pathogenic mechanism for this organism.

CVCs are recognized risks for bacteremia. As many as 1 in every 20 CVCs have been estimated to result in CRBSI, in part, due to microbial colonization and biofilm formation [19]. Biofilms provide a protective mechanism against antimicrobials and host immune response, making CRBSIs particularly challenging to treat [12]. In fact, 80% of chronic and recurrent infections have been reported to be biofilm-related [12] and microbes within biofilms have been reported to be 10–1000 times more resistant to antibiotics than planktonic cells [13]. Consequently, catheter removal is often required in conjunction with administration of high-dose antibiotics for management of CRBSI caused by biofilm-producing organisms, such as the *C. sputigena* isolate from our patient.

As more reports of infections of *Capnocytophaga* spp. have been described, the pathogenic potential of each species has been a topic of research interest. Infection with *Capnocytophaga* spp. leads to loss of mucosal integrity (e.g., destruction of the immunologic barriers at mucosal surfaces) as well as overall immune (dys) function [1]. Moreover, *C. sputigena* has exhibited multidrug-resistance derived from β -lactamase production [20], and reportedly expresses lipopolysaccharides that activate polyclonal B-cells leading to interleukin-1 production from human peripheral monocytes [21]. In this study, we report biofilm formation as another characteristic that may enhance the pathogenicity of *C. sputigena*, particularly in the presence of biomedical hardware that can serve as a nidus for biofilm development and subsequent bacteremia.

In conclusion, *C. sputigena* is a native component of the oropharyngeal microbiota but has the propensity to cause bacteremia. These species are rare pathogens and the repertoire

of pathogenic mechanisms are compounded by the ability to form biofilms. Understanding these characteristics is essential to inform clinical management of *C. sputigena* infections.

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Ethical approval

Per the Human Research Protection Program, the study did not meet the definitions of human subject research and no IRB review/approval was required.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Author contribution

SF, YM, EMS, MMH and AEPM conceived of the presented idea. SF, AvdG, LADN, MRG, MDN, MMH and AEPM conceived and planned the experiments and SF and AvdG carried out the experiments. LADN, MRG and MDN contributed to sample preparation. SF, YM, EMS, MMH and AEPM contributed to the interpretation of the results. SF, YM, MMH and AEPM wrote the manuscript with input from all authors.

Declaration of Competing Interest

The authors report no declarations of interest.

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