

**Title: Rapid Diagnosis of *Capnocytophaga canimorsus* Septic Shock in an Immunocompetent Individual Using Real-Time Nanopore Sequencing**

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**Abstract**

We present a case of *Capnocytophaga canimorsus* septic shock after a dog bite in an immunocompetent individual, where real-time nanopore metagenomic sequencing characterized the microbial agent within 19 hours, with subsequent confirmation using droplet digital PCR. Oral swabs from the dog demonstrated a nearly-identical *C. canimorsus* isolate by sequencing.

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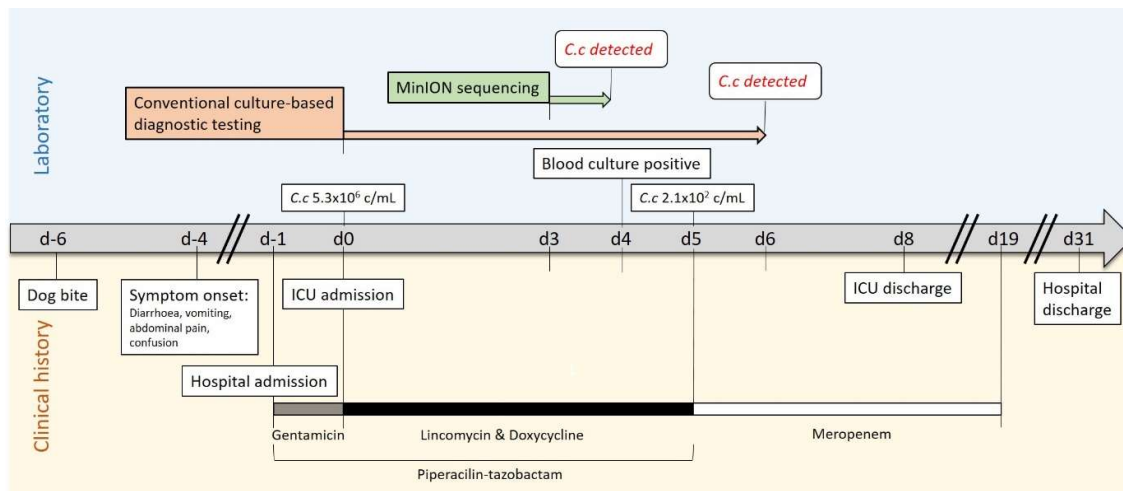
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## Introduction

Bloodstream infection can rapidly progress to septic shock; every hour without appropriate antibiotic therapy increases the risk of mortality while also increasing the length-of-stay in ICU. [Weiss 2014]. Use of broad-spectrum empirical antibiotics whilst awaiting the results of microbiological investigations increases selection pressure for multidrug-resistant organisms. Standard diagnostic methods rely on blood cultures followed by sub-culture, mass spectrometry or other confirmation methods to identify pathogens. Even with typical pathogenic bacteria, the turnaround ranges from 24-47 hours with the use of mass spectrometry [1]. For fastidious organisms and those affected by antibiotic therapy, detection can be further delayed by several days or even result in false negative results [2,3]. Furthermore viruses and some bacteria cannot grow in blood cultures.

To improve clinical outcomes in individuals with suspected bloodstream infection, new rapid diagnostics are essential. One promising approach is to leverage MinION sequencing (Oxford Nanopore Technologies, Oxford, UK) which sequences individual DNA or RNA molecules in an unbiased, real-time approach. The real-time sequence data capability can be coupled with downstream software to characterize the sequences as they are streamed off the instrument and has been demonstrated in a proof-of-concept study through the detection of Chikungunya, Ebola and Hepatitis C viruses in human blood [4], and lower respiratory tract infections [5].

Here we report an unusual case of *Capnocytophaga canimorsus* bloodstream infection where the MinION sequencer, along with droplet digital PCR (ddPCR) were used to rapidly detect and characterize the etiological pathogen (**Figure 1**).



**Figure 1.** Timeline of patient's clinical management progression, including timing of diagnostic testing, bacterial loads (genome copies/mL of blood) of *Capnocytophaga canimorsus* (*C.c*) in whole blood as determined by bespoke droplet digital PCR, and antimicrobial therapy. ICU admission is used as the day 0 reference point.

## Case Report

A 62-year-old immunocompetent woman with no significant medical history presented to a regional emergency department with a three-day history of vomiting, diarrhea, non-specific abdominal pain and confusion. She reported a bite to her hand from her own dog five days prior. She denied alcohol use. She used topical diprosone 0.05% cream for dermatitis and oral esomeprazole 40mg daily for indigestion. On initial examination she was afebrile, with a blood pressure of 90/60 mmHg, heart rate 110 beats/minute, and a Glasgow Coma Score of 15/15. She had right upper quadrant tenderness on abdominal examination and a petechial rash on her face and trunk.

A computerized tomogram of the abdomen demonstrated small volume ascites, marked gallbladder wall oedema, bilateral renal cortical necrosis and bilateral adrenal gland oedema. The spleen was present.

On presentation, her C-reactive protein was 359 mg/L (0-6); prothrombin time 39 seconds (8-14), activated partial thromboplastin time 122 seconds (22-35); serum creatinine 234  $\mu\text{mol/L}$  (50-120); Alanine Transferase 869 IU/mL (0-45); Aspartate Aminotransferase (AST) 1199 IU/mL (0-41). Arterial blood gas examination demonstrated a pH 7.29; pCO<sub>2</sub> 27.7 mmHg; calculated Lactate 4.2 mmol/L and calculated base excess -13.4 mmol/L.

A full blood examination demonstrated a haemoglobin of 156 g/L (115-160); platelets  $13 \times 10^9/\text{L}$  (150-450); white blood cells  $12.3 \times 10^9/\text{L}$  (4.0 -11.0) and neutrophils  $8.7 \times 10^9/\text{L}$  (2.0 – 7.5). There was prominent cytoplasmic vacuolation of the neutrophils, with some of the cells showing intracellular long rod-shaped inclusions (**Supplementary Figure 1**). The patient's peripheral blood film did not demonstrate any Howell-Jolly bodies. HIV serology was negative. Extensive investigations including bone marrow aspirate and trephine did not demonstrate any cause of immunocompromise.

She was initially treated with 3L intravenous fluid infusion; intravenous piperacillin-tazobactam 4.5g and gentamicin 300mg; intravenous hydrocortisone 100mg and cryoprecipitate and fresh frozen plasma infusion. However she developed septic shock requiring intubation, mechanical ventilation and inotrope support. She was transferred to a large tertiary hospital for intensive care management.

Blood cultures collected upon admission to the intensive care unit remained negative after three days. The case was referred for enhanced testing using the MinION sequencer in response to a suspected bacterial aetiology due to the observation of rod-like neutrophil inclusions. Total nucleic acid was extracted from excess EDTA whole blood collected for

routine diagnostic purposes on the day of admission (d0), followed by a 10-minute rapid 1D library preparation (Supplementary Materials). The prepared sample was run on the MinION sequencer for 18 hours, producing 17,552 good quality reads (206.2 Mbases, average read length 11,745bp). Of note, yield was considered atypically low due to suboptimal pairing of library preparation kit and flow cell versions. The majority of reads (98.6%) were of human origin, with top microbial hits (**Supplementary Figure 3**) being for *Toxoplasma gondii* (43 reads, 604,353bp total) and *Capnocytophaga canimorsus* (17 reads, 30,063bp total). Further analyses of the *T. gondii* sequences indicated the detections were probable false positives attributable to likely contamination of the *T. gondii* reference genomes with human DNA (Supplementary Materials).

On day 4, the blood culture became positive, with subsequent sub-culturing showing small, slow-growing colonies with a distinct halo phenotype. On day 6, the sub-culture was identified as *C. canimorsus* by MALDI-TOF (bioMérieux, Australia). Oral Stewart's Media swabs from the offending dog were collected, but no *C. canimorsus* could be isolated due to rapid overgrowth by other commensal species.

Based on the generated *C. canimorsus* sequence data, a hydrolysis probe PCR assay was designed and used as a ddPCR to quantify the *C. canimorsus* bacterial load within the d0 and d5 whole-blood EDTA samples, as well as their associated plasma fractions (Supplementary Materials). The d0 whole blood sample showed approximately 150-fold greater concentration of bacterial load compared to the respective plasma ( $5.33 \times 10^6$  copies/mL and  $3.54 \times 10^4$  copies/mL, respectively), while the d5 whole-blood and plasma showed equivalent, yet markedly reduced bacterial loads ( $2.1 \times 10^2$  and  $2.4 \times 10^2$ , respectively) (**Supplementary Figure 4**). The assay was also used in a real-time PCR format to detect *C. canimorsus* within two oral swabs collected from the dog (Supplementary Materials, **Supplementary Figure 5**).

The patient and dog *C. canimorsus* genomes were sequenced through a combination of MinION and Illumina sequencing, generating complete genomes which were nearly indistinguishable (Supplementary Materials). A computational analysis of virulence markers [6] indicated the patient isolate was of the Serotype D lineage. No antibiotic resistance markers were identified within the patient isolate genome, which fit the fully susceptible antibiotic profile obtained as part of the culture-based diagnostic investigation (Supplementary Materials).

The patient was treated initially with intravenous piperacillin-tazobactam, lincomycin and doxycycline, and subsequently with 14 days of intravenous meropenem (**Figure 1**). A percutaneous cholecystotomy was performed. She required supportive care and several weeks of intermittent haemodialysis, but eventually returned home and remains well.

## Discussion

*Capnocytophaga canimorsus* is a common commensal bacteria of the oral cavities of cats and dogs which can lead to potentially fatal infections through minor bites, scratches or exposure to saliva [7,8]. The fastidious bacteria requires extended incubation times, leading to delays in detection and appropriate treatment [7,9]; thus new diagnostic approaches, such as the rapid nanopore sequencing described in this study, need to be considered.

In our case, the diagnosis of *C. canimorsus* bloodstream infection was considered based on the history of dog bite and the microscopic appearance of rod-like inclusions in the neutrophils. Standard microbiological culture methods were initially negative, leading to the use of MinION sequencing which identified the bacteria in 19 hours, in contrast to the 6.25 days it took for traditional culture-based methods to yield the same diagnosis. Rapid confirmation of the diagnosis allowed reduction of antibiotic use. There was initial concern that the presentation may represent haemolytic-uraemic syndrome consequent to a foodborne

pathogen, and de-escalation of a public health response was possible with determination of the microbiological cause of the presentation.

The use of rapid sequencing and quantification technologies also allowed for additional characterization of the bacterial isolate, confirming the suspected etiological link between the dog bite and clinical presentation. Genomic analyses showed the isolate to be serotype D, which is not commonly found in clinical cases and thus not considered to be highly virulent [6]. The low virulent serovar status is unusual given the patient also did not have significant risk factors for *C. canimorsus* infection, such as immunosuppression, asplenia, heavy alcohol use, or elderly age [8, 10-12]. Apart from  $\beta$ -lactamase production, no additional resistance was detected by culture or molecular methods, consistent with the bacterial load reduction demonstrated by ddPCR, and the patient's clinical response to antibiotic treatment. These results highlight the importance of obtaining blood samples prior to, or early in the application of antibiotics for sequencing-based diagnostic approaches, as the large decrease in bacterial DNA seen on d5 would reduce the effective sensitivity or even lead to outright false negative results with the MinION platform. Additionally, the ddPCR data show the utility of using whole-blood rather than plasma for PCR or sequencing based approaches to sepsis diagnosis given the observed high intracellular bacterial loads. Currently the MinION output formats are still mostly research-centric, however efforts are underway to make the reporting more streamlined and less ambiguous in order to make the platform amenable for use in the routine diagnostic setting.

In summary, *Capnocytophaga canimorsus* should be considered in cases of suspected sepsis involving cat or dog contact, even if the patient does not present with known risk factors. Real-time nanopore sequencing has a demonstrated ability to greatly reduce the time to diagnosis, particularly in cases of atypical infections caused by fastidious organisms, and



shows potential for accurate pathogen characterization to inform clinical and public health management.

**Ethics:** This case report was exempt from The Royal Brisbane and Women’s Hospital Human Research Ethics Committee approval, however the patient’s written consent was obtained to conduct and publish this case study.

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**Conflict of Interest:** LC has received funding from Oxford Nanopore Technologies (ONT) for development of basecalling algorithms and has received ONT funding to cover travel and conference fees to attend an ONT community meeting. LC and SB hold a Queensland Government grant to explore the use of MinION as a diagnostic tool. All other authors report no conflicts of interest.

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