Case Report

A rare case of early-onset neonatal sepsis

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Introduction: *Capnocytophaga sputigena* is a capnophilic Gram-negative bacillus normally residing intra-orally in humans, and may be associated with opportunistic infections in the immunocompromised. A case of early-onset neonatal sepsis is described, which has only rarely been reported as a cause of premature birth and neonatal septicaemia.

Case presentation: This case report focuses on the rare presentation of a microbe, *Capnocytophaga sputigena*, causing pre-term labour, early neonatal septicaemia and maternal chorioamnionitis. To the best of our knowledge, it is the first case report of such a presentation in New Zealand. This case helps to demonstrate the contrast between the stormy clinical course in the newborn and the silent asymptomatic presentations in the mother.

Conclusion: *Capnocytophaga* spp. normally reside in the human oral cavity and have been associated with the pathogenesis of periodontal disease. They have also been reported in wounds in humans inflicted by animal bites, and in a wide spectrum of opportunistic infections in the immunocompromised. Only rarely have *Capnocytophaga* spp. been attributed as a cause associated with pre-term birth, early neonatal septicaemia or chorioamnionitis.

Keywords: antibiotics; chorioamnionitis; CPAP; fever; funiculitis; neonatal sepsis; pre-term birth; respiratory distress; septicaemia.

Case report

A 19-year-old primigravida (gravida 1, para 0) was admitted acutely to hospital because of the sudden onset of pre-term labour at 26 weeks of gestation. She was otherwise well during her pregnancy. Antenatal ultrasounds were performed at 20, 23 and 26 weeks (the last 1 day prior to delivery). Scan reports documented normal anatomy and normal intrauterine fetal growth with a normal amount of amniotic fluid. Membranes ruptured at delivery, with the liquor described as clear. There was no maternal fever and no fetal tachycardia. Antenatal steroids were administered 2 h prior to delivery and intravenous penicillin (1.2 g) 90 min before delivery. The mother gave birth via a normal vaginal delivery to a male baby on the same day of admission.

The male baby weighed 975 g at birth (50 % for 26 weeks). Apgar scores were 4, 4 and 7 at 1, 5 and 10 min, respectively. At delivery, the baby showed minimal respiratory effort. Heart rate dropped to less than 60 beats min^{-1} at which point the baby was intubated and chest compressions commenced with a good response. The newborn was ventilated for 12 h and received continuous positive airway pressure (CPAP) for 22 days. Serial head ultrasound scans, the first on day 5, and magnetic resonance imaging on day 34 of life confirmed a large left-sided cerebellar haemorrhage. All through the initial stages, the baby remained afebrile.

Investigations, including blood tests and blood cultures, were performed on samples from the newborn within the first 24 h of life. His C-reactive protein (CRP) was noted to increase to 18 mg l^{-1} (normal range 0–5), and his full blood count showed raised white blood cell counts of 2.8×10^{10} l^{-1} (normal 8.0×10^{9}–2.5×10^{10}). The neutrophil differential count was 1.37×10^{10} l^{-1} (normal 5.0×10^{9}–2.1×10^{10}), with a mild increase in band neutrophil forms and neutrophil toxic changes in the peripheral smears. The left shift was calculated at 13 %. His mobile chest X-ray revealed opacities in the left hemithorax and right upper lobe, with some volume shift to the left side. The radiological diagnosis was under-ventilation of the right upper lobe and the left lung. An X-ray of the abdomen was normal. Initial antibiotic therapy consisted of penicillin and gentamicin. Blood cultures collected soon after delivery were flagged as positive at 24 h with Gram-negative bacilli. When the blood culture findings were reported to the Neonatal Unit team, penicillin was changed to cefotaxime. At 72 h, with the organism identification, gentamicin was changed to

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Abbreviations: CPAP, continuous positive airway pressure; CRP, C-reactive protein; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

amoxicillin/clavulanic acid after discussion with the regional Paediatric Infectious Diseases Service. Repeat blood cultures collected on days 37, 38 and 40 were culture negative. Despite repeated attempts, a satisfactory cerebrospinal fluid could not be obtained. Urine was not cultured.

Subcultured plates from the baby's positive blood cultures showed growth on Columbia horse blood agar (Fort Richard) and chocolate agar (Fort Richard) incubated in 5 % CO_2 at 37 °C. Colonies were tan coloured with spreading margins and moist centres. The colonies on blood agar plates showed no haemolysis. Microscopy with Gram staining demonstrated long, fusiform-shaped, straight to slightly curved Gram-negative bacilli.

The mother was clinically well with no symptoms or physical signs of infection and she remained afebrile. Investigations on the mother reported that there was a raised white cell count of 2.65×10^{10} l^{-1} (normal 4.0×10^9–1.5×10^{10}) with an increase in differential neutrophil counts of 2.2×10^{10} l^{-1} (normal 3.4×10^9–1.05×10^{10}). There was no significant growth from a mid-stream urine culture. An endocervical swab showed heavy growth of mixed genital flora including *Candida albicans* after culture on blood agar, chocolate agar and a split New York City/Sabouraud dextrose plate (Fort Richard). Moderate growth of tan-coloured colonies of identical appearance to the Gram-negative organism from the infant's blood was also identified. There were no *Neisseria* or *Listeria* spp. isolated.

Swabs taken from the mother's placenta over the fetal sides, between fetal membranes and on the maternal sides revealed growth of yellowish tan colonies similar to those found in the baby's blood culture and mother's endocervical swab.

The isolates were first tested with BioMérieux VITEK MS (MYLA Version 3.2.0_5. Sys. Compliance: VITEK MS 1.1.0), which was able to give a rapid identification, using MALDI-TOF MS. The MALDI-TOF MS test was positive, identifying the organism as *Capnocytophaga sputigena*, with a confidence value of 99.9.

The following biochemical tests were performed on the isolates obtained from the blood cultures, placental swabs and endocervical swab. They showed negative reactions with catalase, oxidase, indole and urease tests. The test for β-galactosidase was positive as it hydrolysed the colourless ortho-nitrophenyl-β-galactoside broth into ortho-nitro-phenol forming a yellow colour. On testing for carbohydrate utilization, there was acid production with lactose, sucrose, maltose and glucose. All these tests were consistent with the biochemical characteristics of *C. sputigena*.

A representative isolate from the blood culture was also identified by the Institute of Environmental Science and Research as *C. sputigena* using 16S rRNA gene sequencing, which showed 99.2 % DNA sequence identity. MICs were determined using E-tests on blood agar incubated in 5 %

CO_2. The amoxicillin MIC was 0.023 mg l^{-1} and the cefotaxime MIC was 0.016 mg l^{-1} after 48 h. The isolate was negative for β-lactamase using a cefinase disc.

The placenta was sent for histology, and the pathology report showed funiculitis, intervillositis and chorioamnionitis in the placenta. However, despite abnormal investigation findings, the mother remained asymptomatic and afebrile, and was discharged home on the day following delivery.

The diagnosis in this case was *C. sputigena* infection with early-onset neonatal sepsis and maternal chorioamnionitis (with funiculitis and intervillositis), resulting in premature labour at 26 weeks of gestation. Clinically, the newborn's condition stabilized and slowly improved over the following 13 weeks. His CRP and white cell counts gradually returned to normal. His chest X-ray cleared. He continued to have no fever. The antibiotics were stopped after 4 weeks on resolution of the *C. sputigena* septicaemia.

However, approximately 2 weeks after cessation of antibiotics, the baby developed a fever (38.9 °C) for 2 days. His CRP then increased to 7 mg l^{-1}, but his white cell and differential counts were normal. A septic screen was carried out, and antibiotics were started empirically with amoxicillin and amikacin due to concern regarding recurrent sepsis and meningitis. The results of investigations of the septic screen, including cerebrospinal fluid examination and PCR for respiratory viruses including influenza A virus, H1N1 2009 influenza A virus, influenza B virus, respiratory syncytial virus, parainfluenza viruses 1, 2 and 3, adenovirus types 1–8, human metapneumovirus (using nasopharyngeal aspirate tested with an AusDiagnostics multiplexed-tandem PCR Respiratory Pathogen Panel) were all negative. His fever subsided after 2 days, and the antibiotics were accordingly stopped.

After 13 weeks, the baby was discharged home, clinically stable, and will be followed up in the Outpatient Clinic. Although his left cerebellar bleed remained, the associated abnormality in left Sylvian fissure had resolved. The CPAP was no longer needed. He gained weight over the last 2 months and, despite his initial very low birth weight, he weighed 2920 g. Nutritionally, the baby did well on oral feeds and was making a slow but steady progress towards recovery.

Discussion

Capnocytophaga is a genus of facultative anaerobic, fastidious, fusiform, non-spore-forming Gram-negative bacilli that normally reside in the oral cavity and are rarely found in the female genital tract. There are nine *Capnocytophaga* spp. (Versalovic *et al.*, 2011), comprising *C. gingivalis*, *C. ochracea*, *C. haemolytica*, *C. sputigena*, *C. granulosa*, *C. leadbetteri*, genospecies AHN8471, *C. canimorsus* and *C. cynodegmi*. The first seven are normally found in the human

oral cavity, whilst the last two are found as dog or cat oral flora.

In this case, the clinical manifestations were peculiar with a prominent absence of symptoms or signs in the mother's apparently silent subclinical chorioamnionitis in contrast to the initial stormy clinical course of early neonatal sepsis in her newborn child.

A literature search for pre-term births in association with *Capnocytophaga* infections in early gestation in pregnancy revealed 22 such cases (Douvier *et al.*, 1999; Howlett *et al.*, 2007; Iralu *et al.*, 1993; Lopez *et al.*, 2010; Mekouar *et al.*, 2012; Mikamo *et al.*, 1996; Vandemeulebrouck *et al.*, 2008) and, up to 2012, there were only three cases of proven neonatal septicaemia with positive cultures of *Capnocytophaga sputigena* isolated from blood.

Our case constitutes the fourth case of proven early neonatal septicaemia, based on the evidence of positive cultures of *C. sputigena* from neonatal blood samples and placental swab samples. This, coupled with the manifestations of neonatal respiratory distress, the abnormal pulmonary chest X-ray findings and the presence of leukocytosis with an increase in white cell counts and neutrophil differential count at birth, all lend support to the diagnosis of early neonatal sepsis.

In culture-negative cases of women with chorioamnionitis and pre-term labour, molecular methods such as 16S rRNA gene PCR and sequencing can be used to identify bacteria such as *Capnocytophaga* spp. However, as this molecular technique is not routinely available, cases of pre-term labour due to *Capnocytophaga* infection may be missed, especially when the presentation of chorioamnionitis is clinically silent and asymptomatic, or when isolates are not recovered from cultures.

Normally, *Capnocytophaga* spp. can be isolated from the oral cavity associated with the development of periodontitis (Hansen *et al.*, 1996; Mikamo *et al.*, 1996) in immunocompetent persons. They have also been described as responsible for a wide variety of infections (Bonatti *et al.*, 2003; Howlett *et al.*, 2007; Lopez *et al.*, 2010; Mekouar *et al.*, 2012; Vandemeulebrouck *et al.*, 2008; Versalovic *et al.*, 2011), such as septicaemia, meningitis, endocarditis, or wound and chest infections, especially in those with immunodeficiency.

Capnocytophaga spp. are thought to reach the uterus either by ascending through the cervix after orogenital contact from a partner with periodontal disease (Hansen *et al.*, 1996; Mikamo *et al.*, 1996) or by haematogenous spread to the placenta, resulting in sepsis in the fetus.

Usually, Group B streptococci or *Escherichia coli* are responsible for chorioamnionitis and infection of the amniotic fluid through ascending infection after the membranes have ruptured, whereas *Bacteroides* spp., genital mycoplasmas or *Ureaplasma* spp. are responsible for chorioamnionitis in pre-term labour with intact membranes (Goldenberg *et al.*, 2000; Tita & Andrews, 2010). *Capnocytophaga* spp., such as *C. sputigena*, are found only rarely in cases of pre-term labour associated with chorioamnionitis and neonatal sepsis.

Virulence factors of *Capnocytophaga* spp. (Huck *et al.*, 2011; Sbordone & Bortolaia, 2003; Spratt *et al.*, 1995) have been detected from isolates of patients with periodontitis or oral lesions. These virulence factors, consisting of enzymes produced by *Capnocytophaga* spp. (e.g. aminopeptidases and proteases), can cause destruction and damage to periodontal tissues, with production of inflammatory molecules. Hypothetically, the production of inflammatory cytokines may result in their entry into the blood stream, leading to the development of chorioamnionitis.

Capnocytophaga spp. are usually susceptible (Mekouar *et al.*, 2012; Versalovic *et al.*, 2011) to macrolides (e.g. erythromycin and azithromycin), lincosamides (e.g. clindamycin), carbapenems, third-generation cephalosporins, tetracyclines and fluoroquinolones. However, it has been noted that *β*-lactamase can be produced by some *Capnocytophaga* spp., which may then be resistant to penicillins or cephalosporins (including third-generation cephalosporins), and this resistance to *β*-lactams has been reported in patients with leukaemia or neutropenia, associated with *β*-lactamase-producing *Capnocytophaga* spp. (Gomez-Garces *et al.*, 1994; Sébastien *et al.*, 1999). *Capnocytophaga* spp. are usually resistant to aminoglycosides (e.g. gentamicin, amikacin and tobramycin) and colistin.

Conclusion

Capnocytophaga spp. normally reside in the human oral cavity and have been associated with the pathogenesis of periodontal disease. They have also been reported in the wounds in humans inflicted by animal bites, and in a wide spectrum of opportunistic infections in the immunocompromised. Only very rarely are *Capnocytophaga* spp. attributed as a cause associated with pre-term birth, early neonatal septicaemia or chorioamnionitis.

Acknowledgements

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