

Bacteroides and *Hafnia* Infections Associated With Chorioamnionitis and Preterm Birth

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Abstract

A pregnant woman presented with no signs of infection and gave birth by emergency caesarean section at 27 weeks. Placental histopathology demonstrated moderate histological chorioamnionitis. Microbiology cultures from swabs from the mother's vagina during labor were reported negative; however, culture-independent analyses returned extensive *Bacteroides* spp. and *Hafnia* spp. infections. The case illustrates the importance of testing with non-culture methods for emerging genital infections in pregnancy.

Keywords: Pregnancy; Infection; Preterm birth; *Bacteroides*; *Hafnia*

Introduction

Infections of mothers and their babies (both in utero and ex utero) are a major global health challenge.” [1]. Preterm birth (PTB) is the second largest direct cause of child deaths in children younger than 5 years [2], a major cause of perinatal mortality and serious neonatal morbidity, and moderate to severe childhood and life-long disability in developed and underdeveloped countries [3, 4].

Ascending infections from the lower female genital tract into the intra-amniotic cavity are the cause of important morbidities worldwide [5]. These include preterm birth, prema-

ture rupture of membranes (PROM) and placental abruption in the mother; sepsis and intrauterine growth retardation in the fetus; septicemia and respiratory and neurological disorders in the newborn [6].

Knowledge of infections during pregnancy remains incomplete, including their prevalence, optimal diagnosis, pathogenic mechanisms and host susceptibilities [7]. A critical step to address this knowledge gap is to obtain a complete understanding of the diverse microbial taxa in the female genital tract in health and disease. The case here reported shows the importance of applying highly accurate, robust, and systematic cultivation-independent analyses to identify and characterize the female genital microbiota, in order better to diagnose infections during pregnancy.

Case Report

A 29-year-old woman (gravida 1 para 0) presented to the birth suite at 27 weeks gestation in her first pregnancy with a history of an antepartum hemorrhage. Apart from a urinary tract infection due to *E. coli* diagnosed and treated at 10 weeks gestation, the pregnancy had been uncomplicated prior to presentation. On history, the patient reported onset of bleeding an hour before with loss of 50 mL of fresh red blood per vagina. She complained of tenderness across the uterine fundus and reported that her baby had not moved since the bleeding commenced. There was no history of fever or ruptured membranes.

On examination, she appeared anxious, was afebrile and contracting once every 10 min with contractions lasting 25 s. Abdominal palpation demonstrated a tender abdomen, maximally over the uterine fundus. A singleton pregnancy of longitudinal lie, cephalic presentation and occipito-posterior position was noted. The fetal heart rate was 150 beats/min. The fetal cardiocotocograph trace demonstrated early decelerations with the mild uterine contractions, but normal beat to beat variability. Vaginal examination demonstrated a closed cervix, 50% effaced, soft and posterior. A diagnosis of abruption placenta was made.

A swab was collected from the vagina and a urine sample collected, antenatal Celestone therapy, broad spectrum

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Table 1. Bacterial Genera That Accounted for 96% of All the Taxa Detected in the Vaginal Swab

<i>Bacteroides</i>	<i>Phascolarctobacterium</i>	<i>Lactobacillus</i>	<i>Hafnia</i>	<i>Escherichia</i>	<i>Enterococcus</i>
32.00	2.94	7.15	50.10	2.13	1.74

The numbers indicate the relative abundance in percentage of each genus in the total population.

antibiotic therapy and magnesium sulfate for neuroprophylaxis were administered, and the patient was transferred by ambulance to a tertiary facility able to accept a newborn of 27 weeks. At the tertiary facility, following observation for 4 h, the fetal cardiocotocograph tracing deteriorated further with a late component to the decelerations and loss of beat to beat variability. An emergency caesarean section delivery was undertaken.

The mother remained afebrile during her hospital course and recovered without adverse event from surgery. The baby was admitted to the level 3 nursery and developed a temperature of 38.8 °C, 3 h after birth. Following blood cultures and swabs, the baby was commenced on broad spectrum antibiotic therapy in addition to requiring rescue surfactant therapy and ventilation. The temperature settled over 30 h. Using standard hospital testing, blood cultures and swabs from the baby and the mother's vagina during labor were subsequently reported as negative, including specific testing for group B *Streptococcus*. Histopathology of the placenta demonstrated features consistent with moderate histological chorioamnionitis, with dense infiltration of the chorionic plate and subamniotic tissues by neutrophils. However, no evidence of vasculitis or funisitis of the umbilical cord was noted. At 3-month review mother and baby were doing well. The baby had been discharged home after 12 weeks in hospital and was enrolled in the preterm infant follow-up program.

DNA extraction of the earlier vaginal swab that was preserved cold in a sterile tube was performed using the QIAamp DNA Mini Kit (QIAGEN, Chadstone Centre, VIC, Australia) according to the manufacturer's instructions. The concentration and quality of DNA was measured using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies; Wilmington, DE, USA). Tag-encoded amplicon pyrosequencing analyses were performed at the Research and Testing Laboratory (Lubbock, TX, USA) based upon established and validated protocols. The microbial community was assessed by high-throughput sequencing of the 16S rDNA gene utilizing a Roche 454 FLX instrument with Titanium reagents. The data derived from the high-throughput sequencing process were analyzed employing a pipeline developed at the same laboratory.

Culture-independent analyses of the microbiota returned that the patient had an extensive *Hafnia* spp. infection, in-

cluding a 5.4% content of *Hafnia alvei*, smaller abundance of *Bacteroides* spp. and *Lactobacillus* spp., and negligible presence of other bacteria (Table 1). At the species level of the total bacterial community, 26.1% was *Bacteroides fragilis* and 6.3% *Lactobacillus crispatus*.

The work received ethics approval from the University of Notre Dame Australia Human Research Ethics Committee, registration number 010120S of 18/10/10.

Discussion

Bacteroides fragilis is involved in gynecological infections and pathology; enterotoxigenic strains have been associated with vaginal disease [8]. The bacterium has been associated with intra-amniotic infections [9], spontaneous mid-gestation abortion and PROM [10]. Nonetheless, a relatively high carriage rate of *B. fragilis*, including enterotoxigenic strains, without presenting clinical problems was reported amongst pregnant women in Poland [11]. The impact of vaginal colonization by enterotoxigenic *B. fragilis* and the full spectrum clinical diseases related to it remain to be defined [12].

Phascolarctobacterium spp. are high producers of acetate and propionate short chain fatty acids from fermentation of succinate or lactate, and the genus specializes in the utilization of succinate produced by other bacteria [13]. The detection of bacteria of this genus in the vagina of the patient could be related to the high levels of *B. fragilis* as bacteria of the genus *Bacteroides* are major succinate producers [14].

Lactobacilli are considered normal flora of the vagina [15, 16]. Analyses of microbiome data of healthy women from samples in three regions of the vagina, introitus, mid-region and posterior fornix, showed that the core vaginal microbiome contained only the genus *Lactobacillus*; at variance with other body sites, the relative abundance of this genus was very constant between healthy subjects. Principal component analysis suggested the presence of potentially three microbiome types in the vagina: one in which a single *Lactobacillus* spp. was predominant, and the two most common species were *L. crispatus* and *L. iners* [15]. Another microbiome type had as most abundant taxa of the family Bifidobacteriaceae in 5% of the samples. In the third type of vaginal microbiome, taxa were found of the genera *Atopo-*

bium, *Prevotella* and *Propionobacterium*, as well as of the order Clostridiales [17].

Bacteria of the genus *Hafnia* rarely cause infections in adult humans; most often they colonize the gastrointestinal tract, but also have been isolated from blood, liver abscesses, urogenital and respiratory tracts, and have been the etiological agent of septicemias [18]. The bacterium has been associated with pyelonephritis [19, 20]. Owing to an expression of fewer virulence factors than other members of the Enterobacteriaceae family, their role in causing clinically significant infections outside the gastrointestinal tract needs further clarification. Its comparatively lower virulence makes *Hafnia* infection to occur in immunosuppressed patients or persons with underlying diseases, and in such instances it has been associated and may be responsible for serious nosocomial or community acquired infections [18]. The rate of isolation of *Hafnia alvei* is higher in females, and it is most frequently found in polymicrobial cultures [20]. The bacterium has been isolated in Turkish women with cervicovaginitis suggesting that etiologic diagnosis of this disease is required to take appropriate preventive and therapeutic measures [21].

Hafnia pediatric infections are infrequent, and found exceptionally in neonates. There are several reports of *Hafnia* bacteremias in neonatal intensive care units (NICU) [22-24]. The presence of *Hafnia alvei* in NICU has caused opportunistic infections in premature newborns undergoing diagnostic or therapeutic invasive procedures. Nosocomial polymicrobial sepsis with positive *Hafnia* cultures was diagnosed in four premature neonates with intravenous catheters and parenteral feeding, but without previous abdominal pathology or surgery [22]. In another report, polymicrobial cultures that included *Hafnia* were isolated from the blood of 10 premature infants of gestational ages 24 - 31 weeks. In these neonatal infections, the infants were also subject to invasive procedures [24]. The isolates were resistant to antibiotic treatments with ampicillin and first generation cephalosporins, but susceptible to vancomycin and gentamicin.

The sequencing analyses indicated that 50.1% of the taxa belonged to the genus *Hafnia* of which 5.4% were identified as *H. alvei*. The genus *Hafnia* comprises two species *H. alvei* and *H. paralvei* [25]. The results suggested that the rest of the *Hafnia* taxa belonged to the *H. paralvei* spp. or to new taxa as yet uncharacterized. *Hafnia* spp. have different susceptibilities for various antibiotics; a study with 68 clinical isolates demonstrated that more than half of the strains tested were resistant to tetracycline, ampicillin-sulbactam, and first generation cephalosporins [26], and penicillin, oxacillin and amoxicillin/clavulanic acid [19]. Resistance within the genus to beta-lactams, beta-lactam inhibitor combinations, and cephalosporins indicates that some broad spectrum antibiotics will not be effective against this pathogen. Considering also that *Hafnia* can develop resistance to second and third generation cephalosporins owing to the presence of constitutive and induced beta-lactamases, the empirical treatment to

be administered requires careful consideration.

New analytical methods serve to identify with increasing frequency bacterial communities before rarely observed or unknown to colonize the vagina. Many of these species are capable to infect the intra-amniotic space and produce important morbidities; thus, they are categorized as emerging infectious diseases. This case demonstrated the need for cultivation-independent analyses to detect potentially pathogenic species when standard culture-based techniques are negative. Clinical practice should consider broadening the spectrum of bacteria tested to diagnose maternal infections.

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