CASE REPORT

Potential involvement of *Campylobacter curvus* and *Haemophilus parainfluenzae* in preterm birth

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SUMMARY

A woman presented with prelabour premature preterm rupture of membranes and delivered extremely preterm at 26 weeks by caesarean section. Histopathology of the placenta indicated moderately severe histological chorioamnionitis with dense infiltration of the chorionic plate by neutrophils. Two sets of low and high vaginal swabs were taken from the mother. A set sent for microbiological analysis at the hospital yielded negative results. The second set was analysed employing culture-independent high-throughput sequencing methods and revealed significant infections with *Campylobacter curvus* and *Haemophilus parainfluenzae.* This is the first report of *C. curvus* infection in the female genital tract that has been identified in a woman who delivered preterm. The case supports the need to review the standard culture methods employed for microbial analyses in hospitals.

BACKGROUND

Preterm birth (PTB) is a complex and unresolved public health problem across the globe. A better understanding of the causes of PTB is needed to improve access to effective obstetric and neonatal care. Intra-amniotic infection is a complication of pregnancy for which a *firm causal link* with prematurity has been established (although other factors can cause PTB).[1] Many intrauterine infections follow the ascending route from the vagina[2]; thus, identification of the bacterial communities present in this organ during pregnancy will help to achieve a comprehensive picture of its microbiome that can be exploited to promote health and prevent/combat disease. A case is presented of chorioamnionitis and PTB complicated by *Haemophilus parainfluenzae* and *Campylobacter curvus* vaginal infections, which were detected only by using culture-independent techniques when standard culture-based infection screens were negative. *C. curvus* is an emerging infection encountered infrequently in humans. It has been isolated with low frequency from human gastrointestinal samples, and has not been reported in female genital tract samples.[3] The case showed that current methods of detection of bacterial infections based on culturing microorganisms do not reveal all the microflora present in the female genital tract, and suggested that hospital diagnoses will improve by introducing culture-independent methods as standard procedures to identify infectious agents.

CASE PRESENTATION

A 29-year-old woman gravida 3, para 2 presented to birth suite at 25^{+4} weeks gestation in her third ongoing pregnancy with prelabour premature preterm rupture of membranes. She had been diagnosed with a short cervix at 20 weeks during a routine ultrasound examination. She was subsequently prescribed vaginal progesterone pessaries. Her first pregnancy had been complicated by severe pre-eclampsia with delivery by non-elective caesarean section at 33 weeks. Her second pregnancy had been uncomplicated and a term baby delivered by elective caesarean section at 39 weeks.

On admission, she was administered two intramuscular injections of celestone 11.4 mg 24 h apart, and started on intravenous penicillin, gentamicin and metronidazole. An ultrasound demonstrated a symmetrically grown fetus on the 52nd centile, in breech presentation. At the time of admission, her white cell count (WCC) was 12 000/mL and C reactive protein (CRP) was 2 mg/dL. A vaginal swab was collected for microscopy and culture. The fetal cardiotocograph was reactive with a baseline heart rate of 140 bpm.

INVESTIGATIONS

During the next 5 days the patient remained on antibiotics and had daily vaginal swabs and CRP tests performed. Clinically, she remained well with no symptoms and normal observations. Vaginal swabs remained negative. However, there was a progressive rise in WCC and CRP as shown on table 1.

Following discussions with the patient and paediatric team, a decision was made to deliver the baby at 07:00 on day 9 because of the rising measures of inflammation, despite the absence of signs of clinical chorioamnionitis. A magnesium sulfate infusion was started and the baby delivered by non-elective caesarean section at 26^{+5} weeks.

Table 1 Time-evolution of maternal white cell counts (WCC) and C-reactive protein levels during days 7 and 8 after admission to hospital

Time	WCC (/mL)	CRP (mg/dL)
Admission to day 6	<15 000	<13
Day 7 (morning)	–	24
Day 7 (evening)	–	36
Day 8 (morning)	22 000	47
Day 8 (evening)	–	61
Day 8 (midnight)	24 000	75

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Two sets of low and high vaginal swabs were collected at delivery. One set was sent for standard microscopy and culture, and the second set sent for culture-independent analyses. Standard microbiological tests in the hospital where this patient was treated include culturing of 12 genera of bacteria, and PCR analyses for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. All the microbiological results were negative, including two rounds of PCR analyses for *C. trachomatis*. Our culture-independent analyses yielded the same results for the 12 genera and two species of the standard microbiology tests with the exception of *Escherichia coli* DNA that was identified in the high vaginal swab at an abundance of 0.16%.

The placenta was sent for histopathology, which subsequently demonstrated features consistent with moderately severe histological chorioamnionitis, with dense infiltration of the chorionic plate by neutrophils. However, there was no vasculitis of the umbilical cord or funisitis.

TREATMENT

Postdelivery, the mother remained on triple antibiotic therapy for 48 h. Postnatal hospital course was uneventful. She was discharged on day 5.

The baby was admitted to the level 6 neonatal intensive care units (NICU) for airway support, and developed a temperature of 39.1°C 4 h after birth. The baby appeared septic with pallor and elevated WCC of 28 000/mL. Following blood cultures and swabs, the baby was started on broad-spectrum antibiotic therapy. The temperature settled over 48 h. Blood cultures and standard cultured swabs from the baby and the mother's vagina were subsequently reported as negative, including specific testing for group B *Streptococcus*.

OUTCOME AND FOLLOW-UP

Following a 10-week admission, the baby was discharged and enrolled in a PTB follow-up programme. At review 6 months after delivery, there was ongoing respiratory morbidity but milestones were otherwise appropriate for adjusted postnatal age.

The low and high vaginal swabs from the mother sent for culture-independent analysis showed the presence of significant levels of *H. parainfluenzae* and *C. curvus*, respectively (table 2).

The analyses of the batch of samples that included DNA isolated from this patient identified in other participants four different species of *Campylobacter: C. concisus, C. curvus, C. hominis* and *C. ureolyticus* as well as other *Campylobacter* taxa, grouped under *Campylobacter* spp. The only *Campylobacter* taxon identified in the swabs from this patient was *C. curvus*.

DISCUSSION

This is the first report to demonstrate the presence of *C. curvus* bacteria in the upper vagina of pregnant women. The patient was diagnosed with chorioamnionitis and required emergency early preterm delivery. Also, the bacterium *H. parainfluenzae* was found in the lower vagina in high abundance. Both microorganisms were detected at high concentrations in each respective locus only by using culture-independent sequencing methods.

Table 2 Bacterial genera/species that accounted for over 80% of all the taxa detected in the low and high vaginal swabs

Bacterial genera/species	Low vaginal swab	High vaginal swab
Haemophilus parainfluenzae	56.1	18.2
Haemophilus spp.	13.5	4.1
Terrahaemophilus aromaticivorans	12.6	4.1
Campylobacter curvus	1.4	61.3

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

The genus *Campylobacter* consists of curved or S-shaped Gram-negative, oxidase-positive, microaerophilic bacilli.[4] A number of taxonomic revisions have occurred to the genus, with several species transferred to genus *Helicobacter* or the genus *Arcobacter*.[5]

The genus *Campylobacter* contains now 25 species, 2 provisional species and 8 subspecies,[6] of which *C. coli, C. concisus, C. jejuni* and *C. fetus* have attracted the greatest interest in medical research; there are limited data on the pathogenicity of the remaining species.[5]

Knowledge of the pathogenicity of *C. curvus* is limited. There were only four strains in the original description of the species, of which two were isolated from the oral cavity, one from blood and one from an unknown clinical site.[7] Subsequent reports described the isolation of *C. curvus* from the stools of patients with Guillain-Barré and Fisher's syndrome, although no causal linkage could be established with the disease.[8]

Two prevalence studies support the rarity of *C. curvus* as a bowel pathogen; it was found only in 3 of 48 campylobacters isolated from a series of 1300 human stool samples. In another study of 320 semiliquid stool samples, only one sample was positive for *C. curvus* by 16S rDNA gene PCR analyses.[9]

Also, the bacterium was isolated from blood cultures of a patient with liver abscesses.[10] However, another report suggested it may be an under-represented organism in human pathology; investigations into an outbreak of Brainerd's diarrhoea in the USA reported that 20 strains of *C. curvus* were isolated using microfiltration techniques.[3]

For quite some time, prenatal infections with *C. fetus*, although uncommon, have been recognised as a cause of abortion, intrauterine fetal death and stillbirth,[11] and more recently, also as cause of extremely low weight at birth.[12 13] A literature search did not yield any studies reporting the presence of *C. curvus* in the vagina or uterus of women.

Culture-independent analyses identified also high abundance of *Haemophilus parainfluenzae* in a low vaginal swab. This bacterium can cause significant pathology in many body systems, including the urogenital tract.[14] *H. parainfluenzae* behaves as an opportunistic pathogen; it is an uncommon cause of neonatal sepsis, although neonatal infection due to the bacterium *H. influenzae* is well documented and its incidence has recently been increasing.[15] Both bacteria may cause fulminant neonatal infection, particularly in very low birth weight infants with a death rate of more than 50%,[16] and risk factors for sepsis were present in the reported cases of early-onset infection.[14]

Both *C. curvus* and *H. parainfluenzae* are difficult to grow in culture, and their incidence may be under-reported owing to lack of proper and standardised culture techniques, and difficulties associated with species identification. In cases where cultures are negative, diagnosis can be established by molecular diagnostic methods such as broad range PCR and sequence analysis of the bacterium 16S rDNA gene.[17]

In the case presented, the onset of early neonatal sepsis may have been secondary to ascending infection through ruptured membranes, as *C. curvus* and *H. parainfluenzae* were identified in the vagina of the mother. The infant had several risk factors for early neonatal sepsis: low birth weight, prematurity, prolonged rupture of membranes and subsequent histological evidence of chorioamnionitis. In a routine microbiology laboratory,

C. curvus will be missed in cultures under standard conditions because it requires hydrogen to grow, and *Haemophilus* spp. will be missed in cultures with only horse blood agar.[16]

It is not possible to determine definitively whether *C. curvus*, *H. parainfluenzae*, both or neither species of bacteria were the cause of the chorioamnionitis and newborn infection. Blood culture has been the gold standard for diagnosing bacterial sepsis. However, blood cultures in infants who appear to have bacterial sepsis are often negative, which underscores the difficulty of documenting a bacterial aetiology of sepsis in the symptomatic immature neonate. This is particularly challenging in cases in which antenatal maternal antibiotic therapy has been administered, which may affect the ability to detect organisms in neonatal blood cultures.[18] It has become routine clinical practice to initiate broad spectrum antibiotic cover if a neonate demonstrates clinical signs and symptoms of sepsis even if cultures are negative.

In those cases where cultures are positive, group B *Streptococcus* and *E. coli* are the most common organisms causing early-onset sepsis in preterm neonates. Although the incidence of clinical genital infections due to *Haemophilus* species is low,[19] this case and others reported support the significance of *H. parainfluenzae* as a neonatal pathogen. Thus, it would be appropriate that the potential presence of *H. parainfluenzae* is considered in all cases of presumed early onset neonatal sepsis.

Learning points

- This is the first report of the potential association of *Campylobacter curvus* with chorioamnionitis that resulted in early preterm birth. *Haemophilus parainfluenzae* was also identified in the vagina of the mother.
- Standard culture-based methods did not detect the presence of either bacterium in vaginal swabs, confirming that these techniques miss organisms that are occasional pathogens in the human reproductive tract.
- As culture-independent approaches gain wider acceptance into clinical practice, the spectrum of pathological organisms during pregnancy that could be involved in chorioamnionitis will expand as well as the frequencies at which they are identified in maternal infections.
- Knowledge of the potential presence of many pathogens would require a review of clinical practices to monitor infections during pregnancy.

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