Case report

A 23-year-old primigravida woman was admitted to the Teaching Hospital of Amiens (Amiens, France) for preterm labour (PTL) at 29 weeks of gestation and intrauterine growth retardation, which were attributed to alcoholism, smoking and gestational hypertension. Pelvic examination revealed cervical dilatation and apparently intact membranes. The patient was afebrile and haemodynamically stable, had no abdominal pain, and had a leukocyte count of 9.5 x 10^3 cells µl^-1. Serological testing of maternal blood for syphilis, human immunodeficiency virus, hepatitis B, Toxoplasma gondii and rubella was negative. The PTL was treated with nifedipine as a tocolytic (10 mg every 15 min during the first hour followed by 20 mg every 12 h).

At this time, a sample taken from the cervix and vaginal exudate showed the presence of abundant Gram-negative rods that did not grow under aerobic conditions, which was in agreement with bacterial vaginosis. The patient was treated with amoxicillin (50 mg kg^-1 per day) for 7 days. One week after completing treatment, her test-of-cure vaginal culture yielded an Escherichia coli isolate producing penicillinase. A co-amoxiclav treatment was carried out for the next 7 days (50 mg kg^-1 per day).

At 37 weeks gestation, a 1630 g live-born girl was delivered by spontaneous vaginal delivery with Apgar scores of 2 (1 min), 6 (5 min) and 8 (10 min). The total body length and the cranial circumference were 42 cm and 31.5 cm, respectively. The intrauterine growth retardation was homogeneous. There was no suspicion of materno-fetal infection on clinical grounds. No sample was taken from the infant and the amniotic fluid at delivery. During admission, the mother was never febrile and did not complain of abdominal tenderness or chills. She was discharged 2 days after delivery without further complications. At a 6-week follow-up, she remained well, with no signs of infection.

After an uneventful observation period, the baby deteriorated at day 6 being febrile (39.1 °C) and hypotonic. On examination, her respiratory rate was 48 breaths min^-1, and her heart rate was 180 beats min^-1. She had no focal neurological signs. Her anterior fontanelle was full but not bulging. Cranial ultrasonography findings were normal. The rest of the systemic examination was unremarkable. Laboratory findings revealed a white blood cell count of 10.1 x 10^9 cells µl^-1, a C-reactive protein level of 108 mg l^-1 and a blood glucose level of 5 mmol l^-1. Lumbar puncture revealed cerebrospinal fluid (CSF) that was cloudy with 1450 white blood cells µl^-1 (98% polymorphonuclear leukocytes and 2% mononuclear cells), an elevated concentration of total proteins (247 mg dl^-1), a low glucose concentration (<0.1 mmol l^-1) and Gram-negative bacilli on direct examination.

On the basis of these CSF findings, intravenous cefotaxime (200 mg kg^-1 daily in three doses) was started for 21 days combined with netilmicin (6 mg kg^-1 daily in one dose) for the first 5 days. The baby made good progress thereafter. A second lumbar puncture performed on day 11 yielded a CSF with 155 white blood cells µl^-1 (20% polymorphonuclear leukocytes and 80% mononuclear cells) and an elevated concentration of total proteins of 220 mg dl^-1. The three blood cultures, which were recovered at days 6, 7 and 12, remained negative after 5
days of aerobic incubation using BacT/ALERT paediatric culture vials and the BacT/ALERT 3D continuous-monitoring automated blood culture system (bioMérieux). Her C-reactive protein level normalized after 15 days of treatment. After 28 days in the hospital, she was discharged home.

Microbiology
Spinal fluid was cultured on sheep blood-supplemented (5%) and chocolate-polyvitex agar plates, which were incubated at 37 °C in aerobic and 5% CO2 atmospheres, respectively. After 5 days, grey convex colonies (1 mm) were seen on the chocolate-polyvitex agar plate. Gram staining showed pleomorphic Gram-negative bacilli with fusiform swellings. Subculture to sheep blood and chocolate-polyvitex agar plates incubated at 37 °C for 4 days under several different atmospheric conditions yielded growth only on the chocolate-polyvitex agar plates incubated under anaerobic conditions or in 5% CO2 atmosphere.

Catalase and indole production were negative. The biochemical characterization, which was performed using API 20 ANA and API 20E strips (bioMérieux) supplemented with yeast extract (bioMérieux), failed. Matrix-associated laser desorption ionization-time of flight MS (Shimadzu) did not give any significant peaks.

The molecular approach was undertaken to allow identification at the species level. Bacterial DNA was extracted as described elsewhere from colonies on chocolate-polyvitex agar plates (Heijs et al., 2007). The bacterial 16S rRNA gene was amplified using the B8F (5′-AGAGTTTGATCMTGGCTCAG-3′) forward primer and the universal U1406R (5′-ACGGGCGGTGTGRC-3′) reverse primer (Heijs et al., 2007). PCR mixtures (25 μl) contained 10.2 mM Tris buffer, 2.3 mM MgCl2, 50 mM KCl, 2% DMSO, 5 mg BSA, 0.2 mM each dNTP, 0.2 mM each primer and 0.5 U Taq DNA polymerase. Amplification used the following program: 95 °C for 5 min; 35 cycles of 94 °C for 1 min, 57.5 °C for 30 s and 72 °C for 4 min; with a final elongation step of 72 °C for 7 min. It yielded an approximately 1300 bp DNA fragment that was subsequently sequenced on both strands. BLAST version 2.210 analysis of the resulting sequence with known 16S rRNA gene sequences identified the isolate as ‘Leptotrichia amnionii’ with a maximal identity of 100% for ‘L. amnionii’ strain CCUG 51846 (GenBank accession number EF218612) (Thilesen et al., 2007).

The antimicrobial susceptibility of the isolate was determined by agar diffusion (Etest; AB Biodisk) on chocolate-polyvitex agar incubated under anaerobic atmospheric conditions at 37 °C for 4 days. As the interpretive breakpoints for Leptotrichia are not currently established, the susceptibility was inferred based on Clinical and Laboratory Standards Institute breakpoints for anaerobic organisms other than Bacteroides fragilis group (CLSI, 2011). It revealed that the isolate was highly susceptible to penicillin G, amoxycillin, cefotaxime, ceftriaxone and metronidazole. Moreover, the screen for β-lactamase activity using a cefinase disk (Becton Dickinson Microbiology Systems) was negative.

Discussion
Meningitis is an important cause of illness and death in infancy. It is more common in the neonatal period (under 28 days) than at any other time. Infections in the neonatal period are frequently divided into ‘early onset’ (the first 5–7 days, implying vertical transmission) and ‘late onset’ (after the first week of life, most often implying nosocomial or community-acquired infection). Early onset neonatal meningitis most frequently occurs in premature babies with low birth weight or in infants born to mothers with obstetric complications (De Louvois, 1994). Microbial invasion of the amniotic cavity, which results from an ascending infection from the cervix, or acquisition of the microbe by passage through a colonized birth canal at delivery, constitute the two main aetiological factors (Goldenberg et al., 2008).

In the newborn, E. coli and Streptococcus agalactiae are the two leading causes of early onset bacterial meningitis (Gaschignard et al., 2011). Reports of anaerobic Gram-negatives were exceedingly rare (Bobitt & Ledger, 1977). Herein, we describe what is believed to be the first neonatal meningitis caused by ‘L. amnionii’, which is a preferential anaerobic bacterium of the female genital flora.

The genus Leptotrichia, which was established in 1879 by Trevisan (Eribe & Olsen, 2008), includes Gram-negative, non-spore-forming, anaerobic rods, with some strains growing aerobically in the presence of CO2 (Hofstad, 1998; Thilesen et al., 2007; Goto et al., 2007). It is placed in the order Fusobacteriales, in the family Leptotrichiaceae. Seven species belong to this genus: Leptotrichia buccalis, Leptotrichia trevisanii, Leptotrichia goodfellowii, Leptotrichia hofstadii, Leptotrichia shahii, Leptotrichia hongkongensis and Leptotrichia wadei (Eribe & Olsen, 2008). ‘L. amnionii’ (Shukla et al., 2002) is not a validly published name (Eribe & Olsen, 2008).

Recent reports have proven the pathogenicity of Leptotrichia spp. in appropriate clinical situations. L. buccalis, L. trevisanii, L. wadei and L. goodfellowii act as opportunists pathogens responsible for bloodstream infections in immunocompromised patients (Eribe & Olsen, 2008; Couturier et al., 2012), whereas ‘L. amnionii’, which frequently co-inhabit the lower genital tract of women with bacterial vaginosis (DiGiulio, 2012; Ravel et al., 2011; Fredricks et al., 2005; Haggerty et al., 2009), can cause infectious complications in obstetric patients during pregnancy (prepartum) or after delivery (post-partum),
and upper genital tract infections in gynaecological patients with predisposing factors.

‘L. amnionii’ was identified in cervical and endometrial specimens collected from women with histologically confirmed endometritis and salpingitis (Haggerty et al., 2009; Hebb et al., 2004). Several cases also revealed its ability to cause chorioamnionitis in women with intact membranes, accounting for PTL or intrauterine fetal demise (Shukla et al., 2002; DiGiulio et al., 2008; Marconi et al. 2011; Han et al. 2009; Gardella et al., 2004). It was reported in peripartum bacteraemia (De Martino et al., 2004), and in a post-partum renal abscess (Thilesen et al., 2007). Moreover, it was described as the causative agent of a tubo-ovarian abscess and a urinary tract infection in immunocompromised non-pregnant women (Gundi et al., 2004; Domann et al., 2003).

Despite its pathogenicity, ‘L. amnionii’ remains an underappreciated cause of infections due to inherent difficulties with conventional laboratory methods. This fastidious species grows preferentially under anaerobic atmospheric conditions on chocolate agar plates after several days of incubation (Shukla et al., 2002). In addition, it is poorly characterized using routine phenotypic laboratory tests due to its poor chemical reactivity. Identification is achieved using molecular techniques that are time-consuming, expensive and technically demanding. In our case report, the presence of Gram-negative rods in the direct examination of the CSF prompted us to prolong the time of incubation, allowing the growth of the bacteria.

In conclusion, ‘L. amnionii’ emerges as a causative agent of fetal and neonatal infections. Its identification in the medical laboratory is often challenging for clinical microbiologists. To improve the isolation of *Leptotrichia* spp. in the CSF from neonates, we recommend microbiologists use chocolate-polyvitex agar plates incubated under anaerobic conditions for at least 7 days.

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**References**


