Jepp Catherine (Orcid ID: 0000-0003-4440-5790)
Yeoh Daniel (Orcid ID: 0000-0002-6284-612X)

# *Ureaplasma urealyticum* meningitis complicated by hydrocephalus in a preterm neonate

**Instructive Case**

**Authors:** Catherine K Jepp[1], David A Foley[1], I-Ly Joanna Chua[2], Jason Kwong[3,4], Matthew S Payne[5,6], Jonathan Davis[7,8], Daniel K Yeoh[1,,9,10]

1. Department of Infectious Diseases, Child and Adolescent Health Service, Perth, Australia

2. Microbiology, PathWest reference laboratory, Nedlands WA

3. Department of Microbiology & Immunology, University of Melbourne, Melbourne Australia;

4. Department of Infectious Diseases, Austin Health, Heidelberg, Australia

5. Division of Obstetrics and Gynaecology, University of Western Australia, Subiaco, Australia

6. Women and Infants Research Foundation, Subiaco, Australia

7. Department of Neonatology, Child and Adolescent Health Service, Perth, Australia

8. Clinical Senior Lecturer, University of Western Australia, Subiaco, Australia

9. Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia

10. National Centre for Infections in Cancer, Peter MacCallum Cancer Centre, Melbourne, Australia

**Acknowledgements:** Microbiological Diagnostic Unit Public Health Laboratory, University of Melbourne at the Peter Doherty Institute for Infection and Immunity

**Corresponding Author:**

Dr Daniel K Yeoh
Infectious Diseases Unit
Perth Children's Hospital
Perth, WA 6009
Australia
Tel: +61 8 6456 5850
email: daniel.yeoh@health.wa.gov.au

**Conflict of interest:** none declared

***Ureaplasma urealyticum*** **meningitis complicated by hydrocephalus in a preterm neonate**

Case history

A male infant was born at 26+3 weeks gestation with birth weight 900g in the context of prolonged rupture of membranes (29 h), maternal sepsis and chorioamnionitis confirmed on placental histopathology. He was intubated and received surfactant for neonatal respiratory distress syndrome soon after delivery. Benzylpenicillin and gentamicin were commenced empirically, and then changed to cefotaxime monotherapy following isolation of *Haemophilus influenzae* from infant ear swab and placental tissue; *Ureaplasma urealyticum* and *Mycoplasma hominis* were also isolated on placental tissue. There was no growth from routine blood culture and initial lumbar puncture was not performed due to clinical instability. Maternal blood cultures were negative. His clinical condition stabilised and he was extubated to continuous positive airway pressure support on day 3 of life. Bilateral grade IV intraventricular haemorrhages were detected on routine head ultrasound on day 7 of life.

On day 10 of life, he developed hypotonia, poor respiratory effort, frequent apnoeic episodes and seizure activity. He was re-intubated and antibiotics were changed to meropenem and vancomycin. Cerebrospinal fluid(CSF) was collected 48 h after antibiotic switch. The sample was heavily blood stained and yielded no growth on routine media. Temporary clinical improvement was observed with initial resolution of seizure activity, followed by recurrence of myoclonic focal seizures on day 18 of life; anti-seizure medications (phenobarbitone and levetiracetam) were commenced.

Nucleic acid amplification tests were ordered on the CSF sample, including 16S rRNA gene polymerase chain reaction (PCR). On day 22 of life, *Ureaplasma urealyticum* was detected on 16S rRNA gene PCR. This was further confirmed by a specific *Ureaplasma urealyticum* PCR. Retrospective PCR testing of a blood sample taken on day 19 of life was equivocal for *U. urealyticum*. A decision was made not to initiate directed *Ureaplasma* spp. treatment as the infant was assessed to be clinically stable.

The infant had ongoing seizure activity and an increasing head circumference. Serial head ultrasound scans reported increasing ventriculomegaly and presumed hydrocephalus (Figure 1).

On day 26 of life he was transferred to a specialist tertiary paediatric centre for neurosurgical intervention. A Rickman reservoir was inserted on the following day. *Ureaplasma* spp. was detected via PCR and cultured on A7 agar from the intra-operative CSF sample.

Intravenous azithromycin, 10mg/kg/dose once daily (for 28 days), was commenced on day 30. On day 33 the CSF remained positive for Ureaplasma; susceptibility data was unavailable at this time. Ciprofloxacin

7.5mg/kg/dose twice daily was added on day 35 (for 23 days). Serial CSF samples taken from the Rickman reservoir remained negative from day 37. Stabilisation in CSF parameters was observed (Table 1). *U. urealyticum* DNA continued to be detected on CSF PCR until day 56 of life. The patient received a total of 23 days of combined treatment with azithromycin and ciprofloxacin. At the time of completion of the azithromycin/ciprofloxacin course on day 58, a ventriculoperitoneal shunt was inserted.

There was no seizure activity beyond day 29 of life; one day prior to commencing the course of targeted antimicrobial therapy. The patient was discharged home on day 96 of life. He did not develop retinopathy of prematurity and had no oxygen requirement at discharge. At follow up at 18 months corrected gestational age the patient had right hemiplegic cerebral palsy and global developmental delay; he was able to sit independently but had not started to crawl. He has ongoing multidisciplinary developmental follow up.

## Microbiology

The *U. urealyticum* isolate cultured from CSF sample on day 33 underwent whole genome sequencing and assessment for resistance, comparing to the reference sequence isolate *U. urealyticum* ATCC 33699 genome (GenBank CP001184.1) and by searching for known resistance mechanisms in existing antibiotic resistance databases(1). No mutations associated with resistance were observed. Phenotypic susceptibility testing was performed using micro-broth dilution (2). The isolate was phenotypically susceptible to doxycycline (0.125mg/L) and azithromycin (2mg/L) based on CLSI breakpoints, but was resistant to ciprofloxacin (4mg/L); however, it should be noted that as no breakpoints for ciprofloxacin exist for *U. urealyticum*, those for moxifloxacin/levofloxacin were used (≥4mg/L)(2).

## Discussion

*Ureaplasma* spp. have been linked to a broad spectrum of neonatal diseases including bronchopulmonary dysplasia, retinopathy of prematurity and necrotizing enterocolitis (3). Many of these associations are based on observational studies without proof of direct causation (3). *Ureaplasma* spp. meningitis is rare but has been described in both term and preterm infants (4). It is associated with intraventricular haemorrhage, hydrocephalus and developmental disability (5).

In retrospect, directed therapy for Ureaplasma spp. meningitis could have been considered earlier in this case. A number of factors contributed to the initial delay in detection, recognition of significance and initiation of treatment, highlighting the challenge in diagnosing *Ureaplasma* spp. meningitis in neonates. Firstly, *Ureaplasma* spp. will not be detected using conventional culture media or standard CSF NAAT

panels and targeted testing is required (4). In this case, *Ureaplasma* spp. was unexpectedly detected on 16S rRNA gene sequencing. This test is labour intensive, typically has a slower turnaround time and, to facilitate generic amplification, is less sensitive than a targeted nucleic acid amplification test. As the CSF sample was heavily blood stained; it was unclear if detection was representative of sampling from the peri-meningeal space. In addition, *Ureaplasma* spp. detection in blood and CSF can occur in the absence of symptoms (5). Furthermore, seizures, intraventricular haemorrhage and hydrocephalus have a number of alternative aetiologies in this age group (6). As the patient had clinically stabilized at the time the 16S rRNA gene result became available, treatment was not initiated at that point. The subsequent progression of symptoms together with placement of a CSF reservoir (facilitating repeat sampling to demonstrate persistent detection of *Ureaplasma* spp.) provided a clear rationale to start targeted treatment. A diagnosis of *Ureaplasma* spp. meningitis was considered most likely because of the combination of intraventricular haemorrhage, hydrocephalus, seizures and persistent detection of *Ureaplasma* spp. on CSF culture(5).

Even in confirmed cases of *Ureasplasma spp* meningitis, optimal treatment is not well defined. Macrolides are the antibiotic of choice for *Ureaplasma* spp. infection outside of the central nervous system (CNS) in neonates (7), however CSF penetration of macrolides may theoretically limit efficacy in CNS infection (5). Although azithromycin, a newer macrolide, has superior CNS penetration compared with erythromycin, CSF penetration is poor and may in this case have contributed to persistent culture positivity following treatment initiation (8). Combination therapy for *Ureaplasma* meningitis has been advocated by some given poor CSF penetration of macrolides, the challenges with susceptibility testing and increasing rates of macrolide resistance in some parts of the world (5, 9). In this case, as culture remained positive 3 days after commencement of azithromycin, a quinolone was added. There is no consensus on optimal duration of therapy but a minimum of 14 days has been suggested (1, 3).

There is limited availability of phenotypic susceptibility testing for *Ureaplasma spp.* in diagnostic laboratories. In this case genotypic and phenotypic testing was performed at a research laboratory. Culture independent methods such as whole genome sequencing and *in silico* resistance testing may have a role in predicting antimicrobial susceptibility testing of uncommon organisms in the future. For this isolate, the genotype correlated with phenotypic susceptibility testing for macrolides and doxycycline. No mutations were identified that predicted fluoroquinolone resistance. The limitations of such genotypic approaches must be acknowledged.

## Conclusion

We present the case of a preterm neonate with intraventricular haemorrhage and hydrocephalus associated with *Ureaplasma* spp. meningitis, successfully treated with macrolide and quinolone therapy.

This case highlights the potential for *Ureaplasma* spp. to cause invasive neonatal infection and serves as a reminder to consider *Ureaplasma* spp. as a pathogen, especially in cases of 'culture negative' meningitis. The involvement of paediatric infectious disease specialists may be helpful in navigating the uncertainty about the pathogenic role of this bacteria as well as choice of antibiotics and duration of treatment.



Learning points

1) *Ureaplasma* spp. meningitis is rare but has been described in preterm neonates and, less frequently, term neonates. It is associated with intraventricular haemorrhage, hydrocephalus and long-term sequelae, including developmental disability.
2) *Ureaplasma* spp. will not be detected using conventional culture media. Cerebrospinal fluid nucleic acid amplification (e.g. PCR) coupled with targeted microbiological culture is required.
3) Optimal treatment for *Ureaplasma* spp. meningitis is unclear. Dual therapy is advocated by some experts.
4) There is limited availability of phenotypic susceptibility testing in diagnostic laboratories. Genotypic resistance testing is becoming a more viable alternative, but results need to be interpreted with caution.

References

1. Su M, Satola SW, Read TD. Genome-Based Prediction of Bacterial Antibiotic Resistance. J Clin Microbiol. 2019;57(3):e01405-18.

2. Beeton ML, Chalker VJ, Maxwell NC, Kotecha S, Spiller OB. Concurrent titration and determination of antibiotic resistance in ureaplasma species with identification of novel point mutations in genes associated with resistance. Antimicrobial agents and chemotherapy. 2009;53(5):2020-7.

3. Viscardi RM. Ureaplasma species: role in neonatal morbidities and outcomes. Archives of disease in childhood Fetal and neonatal edition. 2014;99(1):F87-92.

4. Glaser K, Speer CP. Neonatal CNS infection and inflammation caused by Ureaplasma species: rare or relevant? Expert Rev Anti Infect Ther. 2015;13(2):233-48.

5. Clifford V, Tebruegge M, Everest N, Curtis N. Ureaplasma: pathogen or passenger in neonatal meningitis? The Pediatric infectious disease journal. 2010;29(1):60-4.

6. Linder N, Haskin O, Levit O, Klinger G, Prince T, Naor N, et al. Risk factors for intraventricular hemorrhage in very low birth weight premature infants: a retrospective case-control study. Pediatrics. 2003;111(5):e590-e5.

7. Gwee A, Curtis N. Ureaplasma--Are you sitting comfortably? The Journal of infection. 2014;68 Suppl 1:S19-23.

8. Jaruratanasirikul S, Hortiwakul R, Tantisarasart T, Phuenpathom N, Tussanasunthornwong S. Distribution of azithromycin into brain tissue, cerebrospinal fluid, and aqueous humor of the eye. Antimicrob Agents Chemother. 1996;40(3):825-826.

9. Boujemaa S, Mlik B, Ben Allaya A, Mardassi H, Ben Abdelmoumen Mardassi B. Spread of multidrug resistance among Ureaplasma serovars, Tunisia. Antimicrob Resist Infect Control. 2020;9(1):19.

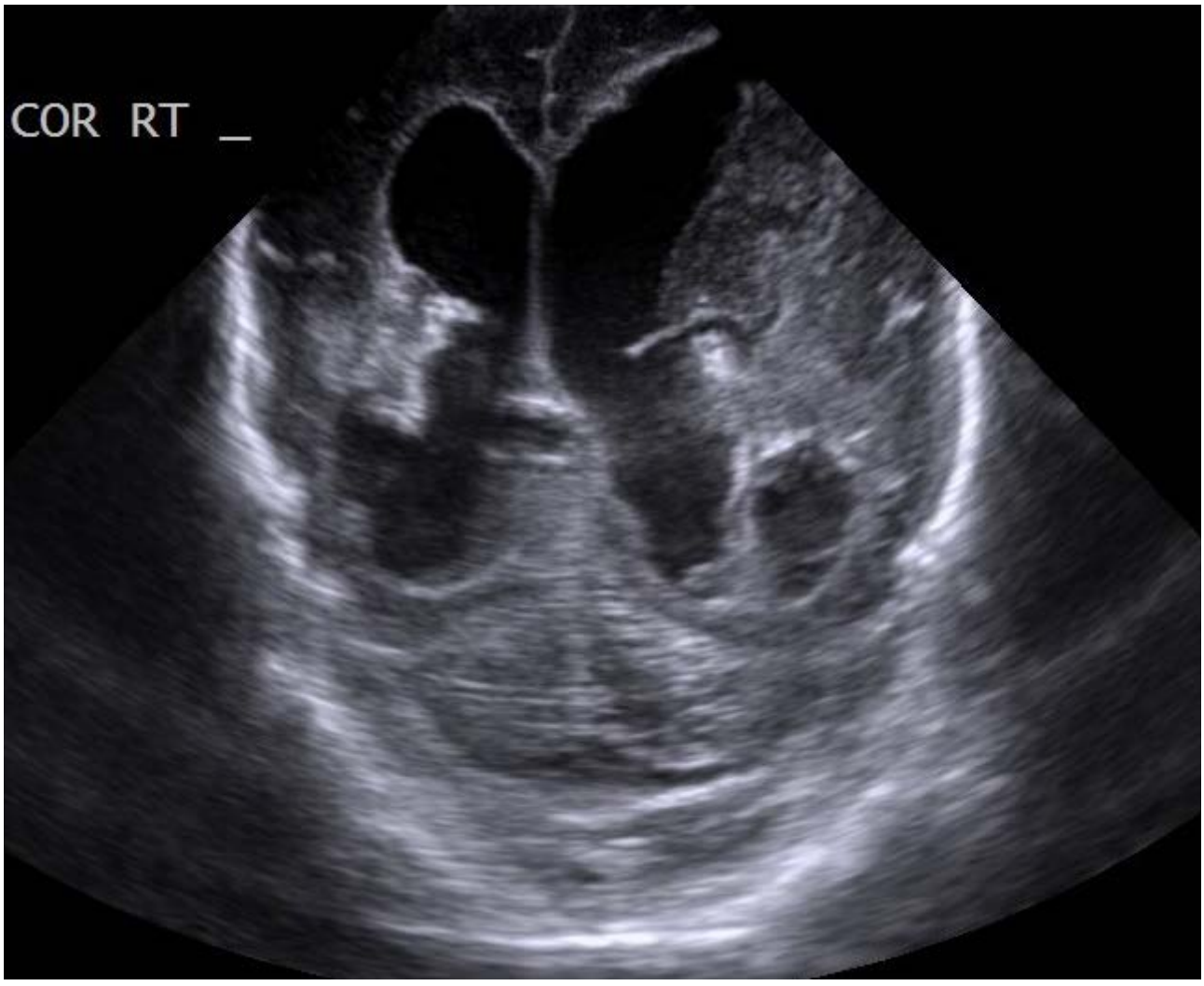


**Figure 1: Cranial ultrasound on day 18 of life, coronal view:** evolution of bilateral grade 4 haemorrhages with moderate to severe bilateral ventricular dilatation and midline shift to the right

**Table 1:** Cerebrospinal fluid result by day of life. UD = undifferentiated, (-) = test not performed, NG = no growth, Pos = positive. [$] = treatment started, [&] = ciprofloxacin added when culture available

| Day | Leucocyte count x$10^6$/L (% Mononuclear) | Erythrocyte count x$10^6$/L | Protein (0.2-0.75 g/L) | Glucose (2.4-4.9 mmol/L) | Culture | PCR Result |
|---|---|---|---|---|---|---|
| **12** | - | Clotted | >3 | 2.2 | - | Pos |
| **27** | - | Clotted | 4.61 | <0.5 | Moderate Growth | Pos |
| **30[$]** | 605 (15%) | 29,700 | 4.34 | <0.5 | - | - |
| **33[&]** | 256 (UD) | 78,300 | - | - | Abundant Growth | Pos |
| **37** | - | Clotted | - | - | NG | Pos |
| **41** | 30 (UD) | 91 | 3.62 | 0.6 | NG | Pos |
| **44** | - | Clotted | - | - | NG | Pos |
| **48** | 16 (UD) | 4,500 | 3.37 | 0.7 | NG | - |
| **51** | 28 (98%) | 5,760 | 3.33 | 0.8 | NG | - |
| **54** | 28 (UD) | 17,700 | 4.01 | - | NG | Pos |
| **56** | 50 (98%) | 288,800 | 4.03 | 0.9 | NG | Pos |

# *Ureaplasma urealyticum* meningitis complicated by hydrocephalus in a preterm neonate

**Instructive Case**

**Authors:** Catherine K Jepp[1], David A Foley[1], I-Ly Joanna Chua[2], Jason Kwong[3,4], Matthew S Payne[5,6], Jonathan Davis[7,8], Daniel K Yeoh[1,,9,10]

1. Department of Infectious Diseases, Child and Adolescent Health Service, Perth, Australia

2. Microbiology, PathWest reference laboratory, Nedlands WA

3. Department of Microbiology & Immunology, University of Melbourne, Melbourne Australia;

4. Department of Infectious Diseases, Austin Health, Heidelberg, Australia

5. Division of Obstetrics and Gynaecology, University of Western Australia, Subiaco, Australia

6. Women and Infants Research Foundation, Subiaco, Australia

7. Department of Neonatology, Child and Adolescent Health Service, Perth, Australia

8. Clinical Senior Lecturer, University of Western Australia, Subiaco, Australia

9. Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia

10. National Centre for Infections in Cancer, Peter MacCallum Cancer Centre, Melbourne, Australia

**Acknowledgements:** Microbiological Diagnostic Unit Public Health Laboratory, University of Melbourne at the Peter Doherty Institute for Infection and Immunity

**Corresponding Author:**

Dr Daniel K Yeoh
Infectious Diseases Unit
Perth Children's Hospital
Perth, WA 6009
Australia
Tel: +61 8 6456 5850
email: daniel.yeoh@health.wa.gov.au

**Conflict of interest:** none declared