*British Journal of Medicine & Medical Research*
*4(33): 5293-5297, 2014*

**SCIENCEDOMAIN *international***
*www.sciencedomain.org*

# *Acinetobacter* Species Associated with Spontaneous Preterm Birth and Histological Chorioamnionitis

**Julie A. Quinlivan[1], Nadeem O. Kaakoush[2] and George L. Mendz[3*]**

*[1]Institute for Health Research, Fremantle, The University of Notre Dame Australia, Fremantle, WA, Australia.*
*[2]School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Kensington, NSW, Australia.*
*[3]School of Medicine, Sydney, The University of Notre Dame Australia, Darlinghurst, NSW, Australia.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors JAQ and GLM collected the swabs, and contributed to the analysis of results and the writing of the manuscript. Author NOK extracted the DNA, and contributed to the analysis of results and the writing of the manuscript.*

***Case Study***

***Received 13th June 2014***
***Accepted 15th July 2014***
***Published 23rd July 2014***

## ABSTRACT

**Aim:** Preterm birth is a complex and unresolved public health problem across the globe. Infection is a factor for which a causal link has been established with preterm birth. A better understanding of its aetiology is required to improve obstetric and neonatal care. The case highlights the limitations of current obstetric hospital microbiology tests, and contributes to the knowledge of bacterial pathogens in the female genital tract associated with preterm birth.
**Case Presentation:** A woman presented with no signs of infection and spontaneously delivered preterm at 34 weeks gestation. Culture-based microbiological results from blood samples and swabs of mother and child were negative. Postpartum histopathology of the placenta demonstrated chorioamnionitis, and vasculitis of the umbilical cord. Cultivation-independent PCR analyses showed a massive *Acinetobacter* spp. infection.
**Conclusion:** Cultivation-independent PCR analyses may detect potentially pathogenic species when standard culture-based techniques are negative. The frequency of

**Corresponding author: Email: QuinlivanJ@ramsayhealth.com.au, N.Kaakoush@unsw.edu.au, George.Mendz@nd.edu.au;*

*Acinetobacter* spp. infections during pregnancy and in neonatal units manifests the need to develop appropriate diagnostic methods that can become standard practice in hospitals and clinics.

*Keywords: Acinetobacter; infection; pregnancy; preterm birth.*

## 1. INTRODUCTION

Preterm birth (PTB) is associated with perinatal mortality contributing to more than two-thirds of all perinatal deaths. Premature delivery is the second largest direct cause of child deaths in children younger than 5 years [1], a major cause of perinatal mortality and serious neonatal morbidity, and moderate to severe childhood disability in developed and under developed countries [1]. It places a burden on society owing to its potential impact on families, health care services and education systems.

Preterm birth may occur owing to maternal or fetal disease leading to induction of labour or elective Caesarean section prior to 37 weeks gestation [2]. Also, it may occur spontaneously or as a result of prelabour preterm prolonged rupture of membranes [2]. Other risk factors of PTB include smoking, multiple pregnancy, especially in association with IVF, viruses and various types infections [2-5]. The aetiology of PTB is multifactorial, but a leading cause of spontaneous PTB is infection resulting from bacterial invasion of the amniotic cavity [2].

The gold standard for identification of intra-uterine infection has been the isolation of microbes from the amniotic fluid using culture-based techniques. However, investigations of the intra-uterine flora of women giving birth preterm based on non-cultivation PCR analyses have demonstrated the presence of pathogenic microflora belonging to more than 50 bacterial genera even in situations without any signs of infection [6]. A case is presented of an *Acinetobacter* spp. associated with spontaneous PTB and histological chorioamnionitis that underlines the need to consider new methods to detect infections during pregnancy.

## 2. PRESENTATION OF THE CASE

A 38-year-old woman (gravida 2 para 1) presented at 34 weeks gestation to the birth suite in spontaneous preterm labour. She had been diagnosed with gestational diabetes mellitus (GDM) following a 75g glucose load at 28 weeks gestation and required treatment with diet and insulin 10 unit protophane nocte. Otherwise, the pregnancy had been uncomplicated with no evidence of fetal macrosomia. The woman's prior pregnancy was uncomplicated with a vacuum delivery of a term male infant of 3720g. On history, the patient reported onset of contractions four hours earlier and had presented because the contractions increased in strength and duration. There was no history of bleeding, fever or ruptured membranes. On examination she appeared well, was afebrile, and contracting 4 times every 10 minutes, with contractions lasting 40s. Abdominal palpation demonstrated a non-tender abdomen with a singleton pregnancy of longitudinal lie, cephalic presentation and left occipito-anterior position. The fetal heart rate was 142. The fetal cardiotocograph trace was normal. Vaginal examination demonstrated cervical dilation of 5cm, intact membranes, station minus 1, and confirmed the abdominal palpation of the fetal position. A diagnosis of preterm labour was made based on established criteria [7,8]. A swab was collected from the vagina as well as a urine sample, and both were sent for microscopy and culture. She was commenced on amoxicillin as antibiotic prophylaxis for Group B *Streptococcus* as her status was unknown

and per hospital policy. She rapidly proceeded to full dilation, spiking a single temperature of 38.8ºC. Her antibiotic regimen was broadened to cover Gram-negative organisms with cefazolin 1g daily, Flagyl 500mg bd and Gentamicin 120mg bd as intravenous bolus doses. Rapidly, she proceeded to vaginal delivery of a female infant that weighed 2250g. Third stage was normal. The mother had no further episodes of fever in her hospital course. The baby was admitted to the level 2 nursery for airway support, and 6h after birth developed a temperature of 38.6ºC. Following blood cultures and swabs, the baby was commenced on broad spectrum antibiotic therapy. The temperature settled over the next 48h. Blood cultures and swabs from the baby and from the mother's vagina during labour were subsequently reported as negative, including specific testing for Group B *Streptococcus.* Histopathology of the placenta demonstrated features consistent with moderately severe histological chorioamnionitis, with dense infiltration of the chorionic plate and subamniotic tissues by neutrophils. There was evidence of vasculitis of the umbilical cord but no funisitis was noted. At 6-week and 3-month reviews mother and baby were doing well. The baby had no apparent abnormalities and displayed normal milestones.

DNA extraction from a second vaginal swab was performed using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. The concentration and quality of DNA was measured using a Nanodrop ND-1000 Spectophotometer (Nanodrop Technologies; Wilmington, USA). The microbial community was assessed by high-throughput sequencing of the 16S rDNA gene utilising a Roche 454 FLX instrument with Titanium reagents. Tag-encoded amplicon pyrosequencing analyses were performed at the Research and Testing Laboratory (Lubbock, TX, USA) based upon established and validated protocols. The sequence data derived from the high-throughput sequencing process was analysed employing a pipeline developed at the same laboratory.

Microbiome analyses returned that the patient had a substantial *Acinetobacter* spp. infection, including a significant content of *A. septicus,* and negligible presence of other bacteria, including normal flora. *Acinetobacter* spp. infection is undetectable by current standard hospital diagnosis methods.

## 3. CONCLUSION

In the last three decades *Acinetobacter* spp. have emerged as important pathogens in nosocomial infections [9]; in particular, as pathogens involved in intra-amniotic infections during pregnancy and in neonatal intensive care units. A number of reports have linked various *Acinetobacter* spp. with adverse pregnancy outcomes and serious neonatal infections [10-12], including three cases of stillbirth in which the mothers had severe *A. septicus* infections [13].

Problems posed by *Acinetobacter* spp. are the multifactorial nature of their virulence [11] and their ability to develop mechanisms of resistance that have resulted in the emergence of multi-drug resistance strains to most commercially available antibiotics [9]; these factors can cause substantial infant morbidity and mortality in untreated infections [14].

In the present case the woman had a number of risk factors for PTB apart from intrauterine infection. These included an age of 38 years and GDM; nonetheless, she had a previous term delivery. A recent systematic review found that older maternal age was associated with preterm birth, but the authors concluded there was insufficient evidence to determine if age was an independent and direct risk factor for PTB, or a risk marker that exerted its influence on gestational age through its association with age-dependent confounders [15]. Gestational

diabetes mellitus is a risk factor for iatrogenic preterm birth, but is a higher risk when diagnosed prior to 24 weeks [16], suggesting that a pre-exisitng or type 2 diabetic state may exist. In the present case, the diagnosis was made at 28 weeks, and insulin was required in management, but the factor responsible for preterm birth was the intrauterine infection.

Notwithstanding the evidence, knowledge of bacterial invasion of the amniotic cavity is at present insufficient to develop effective prevention of infection-related PTB and other morbidities. Cultivation-independent studies to identify and characterise the bacteria that cause intra-amniotic infections associated with histological chorioamnionitis and spontaneous PTB will reveal species that warrant further investigations to elucidate their clinically relevant traits such as virulence mechanisms, evasion of the host immune system, and antimicrobial susceptibilities. This knowledge, in conjunction with the application of improved microbial detection methods in the clinical setting will facilitate the development of new prevention, diagnosis and treatment strategies.

## CONSENT AND ETHICS APPROVAL

The patient consented to a swab for microbial analyses, and to use of results in a deidentified format for research purposes. The work received Ethics approval from the University of Notre Dame Australia Human Research Ethics Committee, registration number 010120S.

## ACKNOWLEDGEMENTS

The study was supported by a grant from the Research Foundation of the Cerebral Palsy Alliance of Australia. NOK is the recipient of a Fellowship of National Health and Medical Research Council of Australia.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: A systematic analysis and implications. Lancet. 2012;379(9832):2162-2172.
2. Goldenberg RL, Culhane JF, Dlams J, Romero R. Preterm birth 1: Epidemiology and causes of preterm birth. Lancet. 2008;371:75-84.
3. Quinlivan JA, Pakmehr S. Fish oils as a population based strategy to reduce early preterm birth. Reprod Syst Sex Disord. 2013;2:116.
   Available: http://dx.doi.org/10.4172/2161-038X.1000116.
4. White SW, Petersen RW, Quinlivan JA. Pandemic (H1N1) 2009 influenza vaccine uptake in pregnant women entering the 2010 influenza season in Western Australia. Med J Aust. 2010;193(7):405-407.
5. Shatrov JG, Birch SC, Lam LT, Quinlivan JA, McIntyre S, Mendz GL. Chorioamnionitis and cerebral palsy. Obstet Gynecol. 2010;116(2,Part1):387-392.
   doi: 10.1097/AOG.0b013e3181e90046.

6. DiGiulio DB. Diversity of microbes in amniotic fluid. Semin Fetal Neonatal Med. 2012;17(1):2-11.
7. Haram K, Mortensen JHS, Wollen AL. Preterm delivery: an overview. Acta Obst Gynecol Scand. 2003;82(8):687–704.
8. Yzeiraj-Kalemaj L, Shpata V, Vyshka G, Manaj A. Bacterial vaginosis, educational level of pregnant women, and preterm birth: A case-control Study. ISRN Infect Dis. 2013;ID 980537:1-4. doi:10.5402/2013/980537.
9. Munoz-Price LS, Weinstein RA. *Acinetobacter* infection. N Engl J Med. 2008;358(12):1271-1281.
10. He M, Kostadinov S, Gundogan F, Struminsky J, Pinar H, Sung CJ. Pregnancy and perinatal outcomes associated with *Acinetobacter baumannii* infection. Am J Perinatol Rep. 2013;3(1):51–56.
11. Pellegrino FL, Vieira VV, Baio PVP, dos Santos RMR, dos Santos ALA, Santos NGB, et al. *Acinetobacter soli* as a cause of blood stream infection in a neonatal intensive care unit. J Clin Microbiol. 2011;49(6):2283-2285.
12. Antunes LCS, Imperi F, Carattoli A, Visca P. Deciphering the multifactorial nature of *Acinetobacter baumannii* pathogenicity. PLoS ONE. 2011;6(8):e22674.
13. Kilic A, Li H, Mellmann, A, Basustaoglu AC, Kul M, Senses Z, et al. *Acinetobacter septicus* sp. nov. association with a nosocomial outbreak of bacteremia in a neonatal intensive care unit. J Clin Microbiol. 2008;46(3):902–908.
14. Visca P, Seifert H, Towner KJ. Acinetobacter infection –an emerging threat to human health. IUBMB Life. 2011;63(12):1048-1054.
15. Newburn-Cook CV, Onyskiw JE. Is older maternal age a risk factor for preterm birth and fetal growth restriction? A systematic review. Health Care Women Int. 2005;26(9): 853-875.
16. Ngai I, Govindappagari S, Neto N, Marji M, Landsberger E, Garry DJ. Outcome of pregnancy when gestational diabetes mellitus is diagnosed before and after 24 weeks of gestation. Obstet Gynecol. 2014;123(Suppl 1):162S-163S.




*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sciencedomain.org/review-history.php?iid=604&id=12&aid=5431*