ORIGINAL ARTICLE

Maternal and neonatal effects of Acinetobacter colonisation in preterm premature rupture of membrane and term labour

Mini Sood, MBBS[1], Zaini Mohd-Zain, DPhil[2], Nor Azizah Abu, MRCPCH[2], Chee Seok Chiong, MMed(Paeds)[3], Noor Shafina Mohd Nor, MB BCh BAO BMedSc[2,4]

[1]Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia, [2]Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh, Selangor, Malaysia, [3]Department of Paediatric, Selayang Hospital, Lebuhraya Kepong Selayang, Batu Caves, Selangor, Malaysia, [4]Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA (UiTM), Sungai Buloh, Selangor, Malaysia

ABSTRACT

Introduction: Some anecdotal reports suggest that maternal colonisation with *Acinetobacter baumannii* during pregnancy is associated with adverse maternal and neonatal effects, including preterm premature rupture of membrane (PPROM). The objective of this study was to compare the maternal and neonatal effects of *A. baumannii* colonisation in cases with PPROM and those with spontaneous onset of labour at term.

Methods: The recruitment of participants' was carried out at Selayang Hospital, Selangor, Malaysia. Vaginal swabs were prospectively taken from 104 patients of PPROM and 111 with spontaneous onset of labour at term. Swabs were also taken from the axillae and ears of their babies. These swabs were cultured to isolate *A. baumannii*. Maternal and neonatal adverse outcomes were documented.

Results: Sixteen mothers were *A. baumannii* positive, eight from each group respectively. None of the cases developed chorioamnionitis or sepsis. Those positive were four cases of PPROM and two babies of term labour. None of the babies developed sepsis.

Conclusions: This study does not support the suggestion that *A. baumannii* colonisation during pregnancy is associated with adverse maternal and neonatal outcomes.

KEY WORDS:
Preterm premature rupture of membrane, Acinetobacter, Neonatal sepsis, Chorioamnionitis

INTRODUCTION

Preterm labour is defined as labour resulting in birth before 37 completed weeks of gestation.[1] The causes for this include infections, multiple pregnancies, and chronic conditions such as hypertension and diabetes. However, no identifiable cause occurs in most cases. The rate of preterm birth ranges from 5% to 18% worldwide.[2]

Preterm premature rupture of membranes (PPROM) is defined as the spontaneous rupture of membranes before 37 weeks of gestation without the onset of labour.[3] It complicates 2% to 4% of singleton and 7% to 20% of twin pregnancies. Antenatally it may result in maternal chorioamnionitis and sepsis. It also causes oligohydramnios leading to foetal pulmonary hypoplasia and skeletal deformities. Preterm deliveries occur in 70 to 90% of cases, leading to neonatal complications, including sepsis, respiratory distress syndrome (RDS), intraventricular haemorrhage (IVH) and death.[4]

Acinetobacter baumannii is a gram-negative non-fermentative coccobacillus.[5] The bacterium is ubiquitous, i.e., present in the water and soil. In humans, it colonises the skin, respiratory and gastrointestinal tracts.[6,7] This bacterium is a significant opportunistic pathogen, causing about 20% of infections in Intensive Care Unit globally and leading to severe complications such as pneumonia and septicaemia.[8]

Some reports suggest that there is an association between *A.baumannii* infection and spontaneous abortion, premature labour, chorioamnionitis and perinatal deaths. However, the reports are limited to case series and reports.[5,8,9] Therefore, the aim of the study was to prospectively determine the maternal and neonatal outcomes of positive cases of *A. baumannii* presenting with PPROM compared to those with spontaneous onset of labour at term.

METHODS

This is a case-control study conducted from March 2012 to February 2015. The cases were enrolled from the Selayang Hospital, Selangor and all laboratory methods were carried out at the Institute of Medical Molecular Biology of University Teknologi MARA (UiTM) Sungai Buloh Campus, Selangor, Malaysia. A total of 108 mothers with PPROM and 111 with spontaneous labour at term were recruited based on convenience sampling from the patient assessment centre and labour room of the Selayang Hospital.

Cases

The inclusion criteria for the maternal PPROM cases were patients between 24 and 36 weeks plus six days of gestation with PPROM, which were diagnosed with a history of leaking liquor that was confirmed by vaginal speculum examination.

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Corresponding Author: Dr Noor Shafina Mohd Nor
Email: drshafina@salam.uitm.edu.my

Those with risk factors for PPROM such as multiple pregnancy, antepartum haemorrhage, cervical incompetence, abdominal trauma and polyhydramnios were excluded.

Controls
The inclusion criteria for the control were term mothers (gestation ≥37) weeks with spontaneous onset of labour with vertex presentation. Those with induced labour, breech presentation, prelabour rupture of membrane (PROM) or elective lower segment caesarean were excluded.

Definition
Chorioamnionitis was defined as infection and inflammation of the foetal membranes, amniotic fluid, foetus or decidua. It was diagnosed if the mother developed fever associated with raised white cell count, purulent cervical discharge or foetal tachycardia.[10]

Neonatal sepsis due to *A.baumannii* was diagnosed if the baby developed tachypnoea, apnoea, tachycardia, bradycardia, poor perfusion or temperature instability, and had positive blood culture for *A.baumannii*.[11]

Sample collection and bacteriology
Following informed written consent, high vaginal swabs were collected by trained medical staff in the perinatal assessment centre (PAC) or labour room during vaginal speculum examinations. All this was performed during the initial assessment of the selected maternal cases. The maternal information was collected in a standard proforma and they were followed up until deliveries. Swabs from the axillae and external ear canal of babies were taken immediately after deliveries.

The swabs were inserted into Amies transport medium with charcoal. The charcoal served to absorb inhibitory substances released in the medium during transportation. The samples were kept in transport containers with ice packs and taken to the laboratory within one hour of collection.

The isolation and identification of the bacteria was performed using standard microbiological technique for vaginal specimen. The swabs were inoculated onto Acinetobacter selective agars (CHROMagar, France) and incubated for 24 hours at 37°C for culture. All red colonies on CHROMagar, suspected of *A.baumannii* were sub-cultured and identified by using the VITEK card (ID-GNA, Biomeriux, USA) on VITEK* 2 automated platform. Antibiotic sensitivity tests were performed using AST-VITEK 2; AST-GN45

Statistical analyses
To the best of our knowledge, there are no studies describing the prevalence of A.baumaniii infection in pregnancy. Therefore, to calculate sample size, we used the following presumptions:

a. *A.baumaniii* infection would constitute a third of all isolates in PPROM (comparable to ICU isolates, reported at 24.85%) and an eighth of isolates in term mothers (comparable to general hospital isolates, reported at 8.2%)[12]
b. The risk of chorioamnionitis from any bacteria is 2-4% in term mothers and 40-70% in PPROM cases.[12]
c. Alpha=0.05, beta=0.2
   This led to sample size of 98 in each group, which we rounded off to 100.

All data was entered and analysed using statistical software, IBM SPSS version 23. All univariate analysis was done using paired t-test for parametric test. Logistic regression analysis was used to compare the outcomes between the two study groups. Level of significance was taken at $p<0.05$.

RESULTS

A total of 108 women with PPROM were recruited during the study period. Out of these, four patients were lost to follow up and hence the swabs from their babies were unavailable for analyses. A total of 111 patients were recruited in the term group. All of their babies were enrolled into the study. There was no significant difference in the demographic characteristics of patients in the PPROM and term group in relation to age, race and nationality (Table I).

The majority of patients with PPROM were between 32-36 weeks (83.3%). There were significantly more primigravida in the PPROM compared to term group (p=0.005). Anaemia was significantly more common in the term group, 30.6% compared to 9% in PPROM group ($p<0.01$). There were also significantly more patients with history of previous preterm labour in the PPROM compared to term group ($p<0.01$) (Table II).

There was no significant difference in the number of mothers positive for *A.baumannii* between the PPROM and term groups [8(7.4%) vs 8(7.2%) respectively]. Methods of delivery were significantly different in both groups with spontaneous vertex delivery occurring in 73.2% of the PPROM cases compared to 94.6% in term patients; and more mothers of the PPROM group had caesarean section compared to term group ($p<0.01$) (Table III).

None of the PPROM group delivered within 24 hours while 87.5% delivered within 24 to 48 hours of rupture of membrane compared to term ($p<0.001$). Apart from that, none of the positive mothers or their babies developed chorioamnionitis or sepsis respectively due to *A.baumannii*. (Table IV) There was also no significant difference in the number of babies positive for *A.baumannii* between the PPROM and term groups [4(50.0%) vs 2(25.0%) respectively].

DISCUSSION

To the best of our knowledge, this is the first study which prospectively compared the maternal and neonatal outcomes of A.baumannii positive cases presenting with PPROM and spontaneous onset of labour at term. Our study showed that 25 to 50% babies of the colonised mothers were positive for *A.baumannii*. There is no existing report on maternal colonisation or the rate of transfer of *A.baumannii* from mothers to their babies for comparison. However, this is higher than 20 to 35% colonisation rate in babies of mothers positive for group B *Streptococcus*, which is the leading cause of neonatal infection acquired during intrapartum period.[13,14]

Table I: Demographic characteristics among PPROM and term groups

	PPROM (N=108) n (%)	Term (N=111) n (%)	p-value
Age[a]			
Mean (SD)	28.14 (5.740)	28.68 (5.505)	NS
Median	28.00	28.00	
Range	15-44	16-48	
Race			
Malay	64 (59.3)	80 (72.1)	
Chinese	24 (22.2)	17 (15.3)	NS
Indian	11 (10.2)	4 (3.6)	
Others	9 (8.3)	10 (9.0)	
Nationality			
Malaysian	100 (92.6)	101 (91.0)	NS
Non-Malaysian	8 (7.4)	10 (9.0)	

[a] years old, SD – standard deviation
NS = not significant

Table II: Obstetric characteristics among PPROM and term groups

	PPROM(N=108) n (%)	Term (N=111) n (%)	p-value
Gestation			
24-27	10 (9.3)	-	
28-31	8 (7.4)	-	
32-36	90 (83.3)	-	
≥37	-	111 (100)	
Gravida			
Primigravida	52 (48.1)	33 (29.7)	0.005
Multigravida	48 (51.9)	67 (70.3)	
Medical Disorder			
Anaemia	9 (8.3)	34 (30.6)	<0.01
Diabetes Mellitus	16 (14.8)	16 (14.4)	NS
Hypertension	2 (1.9)	0 (0.0)	NS
Previous Pregnancy			
History of preterm labour	13 (12.0)	1 (0.9)	<0.01
History of miscarriage	15 (13.9)	21 (18.9)	NS

NS = not significant

Table III: Maternal outcomes in PPROM and term group

	PPROM (N=108) n (%)	Term delivery (N=111) n (%)	p-value
Type of delivery			
SVD	79 (73.2)	105 (94.6)	<0.01
Forceps/ Vacuum	5 (4.6)	5 (4.5)	
LSCS	24 (22.2)	1 (0.9)	
Acinetobacter positive	8 (7.4)	8(7.2)	NS

SVD – spontaneous vertex delivery, LSCS – lower segment caesarian section
NS = not significant

Table IV: Maternal and neonatal outcomes in maternal Acinetobacter baumannii positive

	PPROM n=8 n (%)	Term delivery n=8 n (%)	p-value
Maternal			
Delivery			
< 24 hours	0(0)	8(100)	<0.001
24-48 hours	7(87.5)	0(0.0)	
> 48 hours	1(12.5)	0(0.0)	
Fever	0(0.0)	0(0.0)	NS
Chorioamnionitis	0(0.0)	0(0.0)	NS
Babies			
Acinetobacter positive	4(50)	2(25)	NS
Sepsis	0(0)	0(0)	NS
Hypoglycaemia	2 (25.0)	0 (0.0)	NS

NS = not significant

This study reports that significantly more patients in the PPROM group had LSCS compared to the term group. However, the difference in the mode of delivery cannot be interpreted as due to the presence or absence of *A.baumanni*, but instead this could be due to the nature of PPROM itself with the risk of requiring LSCS is higher than term pregnancy due to various other causes.

None of the mothers or the babies in both groups developed chorioamnionitis or sepsis respectively. This is not consistent with some previous case reports which suggested the association of *A.baumannii* with spontaneous abortion, premature labour, chorioamnionitis and even perinatal deaths.[5,8] However, there no existing study that looked at the maternal colonisation and outcomes in *A.baumannii* positive cases to date, prospectively or retrospectively. Our study provides the first information on a true reflection of insignificant risk of maternal colonisation with *A.baumannii* during pregnancy. Interestingly, we also showed that despite the presence of *A.baumannii* in the eight term patients, their pregnancy reached term without complications, for example preterm labour and neonatal morbidity. Therefore, colonisation with *A.baumannii* does not necessarily harm patients and their offsprings. However, Quinlivon et al., reported a case of *Acinetobacter* spp. infection which resulted in preterm birth and chorioamnionitis. Unfortunately, there was no mention of *A.baumannii* being among the species noted.[15]

However, the numbers of mothers with positive cultures in this study were small (8/104 in PPROM group and 8/111 in term group), so the actual effects of maternal *A.baumannii* colonisation during pregnancy may not be demonstrable from this study. Another possible reason for the good outcome among the positive cases was delivery of the babies within less than 48 hours of membrane rupture in the majority of cases. Shivaraju et al., noted no maternal chorioamnionitis in PPROM cases, wherein majority of the patients delivered within 24 hours.[16]

Our data showed significant differences wherein more babies in the PPROM group were ventilated and had lower birth weight compared to the term group. These are likely attributed to the prematurity of the babies.

The strength of the study was that it involved prospective data collection. All the mothers and their babies were followed up from admission until deliveries and discharge. Two cultures were taken from each baby, which increased the likelihood of isolating the organism. The weakness was that the study did not include the placental and amniotic fluid cultures. Apart from that, this study also did not exclude history of previous preterm labour which is a known risk factor for PPROM, although the number involved was very small.

CONCLUSION

Our study showed no adverse maternal and neonatal outcomes in relation to chorioamnionitis and sepsis due to A.baumannii respectively in both PPROM and term groups. Our study does not support the suggestion that *A.baumannii* colonisation during pregnancy is associated with adverse maternal and neonatal outcomes.

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There is no conflict of interest.

ETHICAL APPROVAL

Ethical approval was obtained from the UITM Ethics committee (600-RMI/ERGS 5/3(60/2011) and Ministry of Health Malaysia Medical Research Ethic Committee (NMRR-11-688-9640).

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