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Colonization of *Acinetobacter baumannii*, *Streptococcus agalactiae* (GBS) and *Candida albicans* in preterm premature rupture of membrane (PPROM) compared to normal labor at term

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ABSTRACT

Aims: Preterm premature rupture of membrane (PPROM) is usually associated with maternal vaginal colonization of Group B *Streptococci* (GBS). However, there are reports on isolation of *Acinetobacter baumannii* in PPROM cases. In order to ascertain *A. baumannii*'s role in PPROM, we determine the colonization of *A. baumannii* and other common vaginal tract flora, i.e. GBS and *Candida albicans*, in women with PPROM, and compared them to those with normal labor at term (NLT). The transmissibility of the organisms to their babies was also investigated.
Methodology and results: A total of 218 high vaginal swabs from 108 and 100 women with PPROM and NLT respectively were collected. The transmission of these organisms to their 215 babies was determined by swabbing the ears and axillae. These were cultured for isolation of *A. baumannii,* GBS and *C. albicans.* Results showed that mothers with PPROM were predominantly colonized with GBS (32.4%), followed by *C. albicans* (19.4%) and *A. baumannii* (7.4%), compared to 10.9%, 17.3% and 7.2% respectively, in women with NLT. Between 34 to 50% of the babies of mothers with PPROM acquired the organisms, with GBS being the most significantly (p=0.000) transferred compared to other organisms. Co-existence of *A. baumannii* with either GBS or *C. albicans,* or both, did not enhance the occurrence of PPROM.
Conclusion, significance and impact of study: Colonization of *A. baumannii* in vaginal tract of pregnant women does not increase the possibility of PPROM, as compared to GBS.

Keywords: Acinetobacter baumannii, *Streptococcuc agalactiae*, *Candida albicans*, Preterm premature rupture of membrane (PPROM), Term labor

INTRODUCTION

Premature rupture of membrane (PPROM) is the rupture of membrane prior to the onset of labor in pregnancy less than 37 weeks of gestation. It is an obstetric complication that can lead to sepsis in neonates since pathogens are frequently acquired during vaginal delivery. Newborns of mothers with risk factors for infections, such as PPROM are 2.3 times more likely to acquire infection compared to those without risk factors (Chan *et al.*, 2013). Colonization of *Streptococcus agalactiae* (Group B Streptococcus, GBS) in the vaginal tract during pregnancy has been recognized to enhance PPROM (Schuchat, 1999). A systematic review, however, showed contradictory findings on the association of GBS with preterm delivery (Valkenburg *et al*., 2009). Furthermore, one report suggested that *A. baumannii* is associated with PPROM, spontaneous abortion and neonatal mortality, but the role of *the organism* in these cases was not discussed (He *et a*l., 2013).

Acinetobacter baumannii is an aerobic, non-motile gram-negative bacillus bacterium that has emerged to be a prevalent cause of hospital-acquired infections (Peleg *et al*., 2008). This bacterium is ubiquitous and able to survive in various environment. It is also capable of adhering on abiotic surfaces as well as in moist devices

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such as ventilators (Yali *et al*., 2014; Lei *et al*., 2016). As an opportunistic organism, it poses a high risk of severe diseases in immunocompromised individuals. In most cases, ventilator-acquired pneumonia ranks the highest infection caused by *A. baumannii* (Garnacho-Montero *et al.*, 2005). Other infections include septicemia and meningitis.

The management of this infection is challenging in view of the emergence of multidrug resistance (MDR) strains leading to longer hospital stay, severe morbidity and even mortality (Zhou *et al*., 2019). A few studies from Iran have documented the emergence of MDR strains in the intensive care and burn units (Bahador *et al*., 2013; Bahador *et al*., 2015; Zarifi *et al*., 2017), whereby the rate of MDR *A. baumannii* in the study was reported to be 56% (Khaledi *et al*., 2016). The emergence of MDR strains has been mainly attributed to previous antibiotic exposures (Zhou *et al*., 2019).

Acinetobacter baumannii is also associated with nosocomial infections among neonates, where outbreaks in neonatal intensive care units are usually attributed to hospital personnel and environmental sources (Huang *et al*., 2002; Melamed *et al*., 2003; Touati *et al*., 2009; McGrath *et al*., 2011). Shete *at al.* reported that the main risk factors associated with *Acinetobacter species* infections are hospital birth, prematurity and birth weight less than 1500 gram (Shete *et al*., 2009).

Natural reservoirs of this bacterium outside hospital environment have not yet been identified, however, a few case reports of its association with complications in pregnant women instigated this study. In rare cases, *A. baumannii* has been reported to cause premature contraction and chorioamnionitis in pregnant women (Aivazova *et al*., 2010; Quinlivan *et al.,* 2014).

Reports on *A. baumannii* being commensals of the vagina are lacking and to the best of our knowledge, the colonization of *A. baumannii* in PPROM mothers has yet to be reported. In this study, we compare the colonization of *A. baumannii* in women with PPROM and normal labor at term (NLT). The presence of other commensals in the urogenital tract of PPROM women, specifically GBS and *Candida albicans*, was investigated as part of this study. The transmissibility of these organisms to their babies was also determined.

MATERIALS AND METHODS

Participants

This study was conducted from March 2012 to February 2015. Maternal PPROM cases included women between 24 and 36 weeks plus 6 days of gestation with PPROM, diagnosed by the history of leaking liquor and confirmed by vaginal speculum examination by obstetricians in Selayang Hospital, Selangor. Exclusion criteria included multiple pregnancies, abdominal trauma, antepartum hemorrhage, cervical incompetence and polyhydramnios. Normal labor at term (NLT) cases were those who had spontaneous onset of labor at gestation ≥37 weeks. Exclusion criteria were premature rupture of membrane (PROM), induced labour, caesarian section and breech deliveries.

Calculation of the sample size was made on the presumption that *A. baumannii* constitutes a third of all isolates in PPROM, based on previous report by Czikk *et al*. (2011). This led to a sample size of 218, which comprised 108 women with PPROM and 110 women with NLT.

A total 215 babies were engaged in this study; 104 and 111 babies (including a pair of twin) born from mothers with PPROM and NLT, respectively. Four babies from mothers with PPROM were not included in this study because they were delivered in different centers.

Ethics

Approval for the study and informed consent were obtained from all subjects in accordance with UiTM Clinical Research Ethics Committee (600-RMI/ERGS 5/3(60/2011) and Ministry of Health Malaysia Medical Research Ethic Committee (NMRR-11-688-9640).

Collection of samples, culture, isolation and identification of microorganisms

Amies Transport Medium with charcoal (Copan, Italy) swabs were used to obtain samples from high vagina of the maternal cases as well as both ears and axillae of their babies. The samples from the mothers were taken upon admission to the perinatal center or labor room, while those from the babies were taken immediately after delivery. All the swabs were cultured within an hour after collection, onto three types of agar; Acinetobacter selective media (CHROMagar) for *Acinetobacter* spp., blood agar for *Streptococcus* spp. and Sabouraud dextrose agar (Oxoid, UK) for *Candida* spp. All suspected *A. baumannii* colonies were identified by VITEK® 2 (Biomeriux, USA) automated instrument, using ID-GNB cards and subsequently confirmed by performing 16s DNA PCR following the methods of Misbah *et al*. (2005). Standard microbiological methods were used to isolate GBS and *Candida* spp. Identification of the isolates to genus level was carried out by using VITEK ID-GP card for gram-positive bacteria and ID-YST card for yeast following the manufacturer's instruction.

Statistical analyses

IBM SPSS version 23 statistical software was used to analyze the data entries. All univariate analysis was done using paired T-test for the parametric test. One-way ANOVA was used to measure the differences among groups with Bonferroni *post hoc* group-wise comparisons. Level of significance was taken at $p < 0.05$.

RESULTS

Out of 218 maternal cases, 59.2% and 35.4% women from PPROM and NLT respectively were positive for at

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least one of the organisms being investigated, with an overall positivity rate of 47.2%. As a whole, GBS (21.6%) was the most common organism isolated, followed by *C. albicans* (18.3%) and *A. baumannii* (7.3%) (Table 1). A similar pattern was observed in PPROM group where GBS (32.4%) constituted the most common isolated organism, whereas *C. albicans* ranked highest in the NLT group. Significantly more GBS (p<0.05) were found in PPROM women compared to those with NLT. There was no significant difference between the two groups in terms of positive cases for *A. baumannii* and *C. albicans.*

Transmission of the organisms from the mothers to their babies was observed to be more frequent in mothers who were colonized with *A. baumannii* (37.5%) compared to GBS (25.5%) and *C. albicans* (30%). Overall, between 34.2 to 50% of mothers with PPROM passed the organisms to their babies, whereas in women with NLT, the transmission was lower (0 to 25.5%). Significantly high number of mothers with PPROM, who were colonized with either GBS (p=0.000) or *C. albicans* (p=0.004), passed the organisms to their babies compared to those with NLT. The transmission of *A. baumannii* however, was not significantly different between the two groups.

The frequency of co-existent colonization of *A. baumannii*, GBS and *C. albicans* in the upper part of the vagina is shown in Table 2. In most maternal cases, the frequency of the organisms co-existing in the same niche was low and there was no significant difference between the two groups. It was interesting to note that a significant number of women with PPROM were colonized with GBS alone compared to those with NLT (p = 0.00).

DISCUSSION

Acinetobacter baumannii has been recognized as one of the major contributors to nosocomial infections. They successfully thrived in health-care environments, causing a wide spectrum of clinical manifestations. In this prospective study, we found that the presence of *A. baumannii* in women in PPROM was low, thus suggesting that this bacterium had no role in PPROM. Furthermore, the results showed no significant difference in the number of *A. baumannii* colonizers between women with PPROM and those with NLT. In comparison to a report by Rani *et al.* (2014), where Acinetobacter colonizers constituted 2% of their PPROM cases, we found higher percentage (7.4%) of positivity for *A. baumannii* in our study. The percentage of *A. baumannii*-positive in their PPROM cases could even be lower than 2% because the species of *Acinetobacter* isolated their study were not identified. Conversely, they reported that *Staphylococcus aureus* and *Escherichia coli* were the commonest organisms to cause PPROM, however, we were unable to concur these findings since our study did not look for these organisms.

About 21% of our maternal cases were GBS colonisers. This is lower than the results obtained in a pilot study conducted in Malaysia, whereby 32% of 56 pregnant women were detected as GBS colonisers (Raj *et al.*, 2009). This finding is higher than reports from other countries; approximately 10% in both Korea (Hong *et al*., 2010) and Hong Kong (Tsui *et al*., 2009), 15% both in India (Chaudhary *et al*., 2017) and Bangladesh (Saha *et al*., 2017) and 20% in Taiwan (Hung *et al.*, 2018). However, our result is within the range of 6.5 to 36% as reported in the European countries (Barcaite *et al*., 2008). Systematic review and meta-analysis on GBS colonization in pregnancy have estimated a prevalence of 17.9% worldwide, of which the prevalence in Southeast Asia is 11.1% (Russell *et al*., 2017).

Amongst the GBS colonizers, the number of women with PPROM (32.4%) were significantly higher than women with NLT (10.9%), which suggests that maternal GBS colonization is a risk factor for PPROM. This finding is similar to a study by Broomand *et al.* (2008) where there were more GBS colonisers in their PPROM group (17%) than the NLT group (4%). Nevertheless, no attempt was made in their study to isolate Acinetobacter, as compared to our study, which has shown positivity for the organism in both groups. This can be explained by the use of selective media to detect *Acinetobacter* spp. in our study. Based on our observation, supported by the studies by Eleje *et al.* (2015) and Shivaraju *et al.* (2015), we hypothesize that *Streptococcus* spp., rather than *A. baumannii*, is the main causative agent for PPROM. Unlike the study of Eleje *et al.* (2015) and Shivaraju *et al.* (2015), we identified the species of the *Streptococcus* isolated as *S. agalactiae.* On the contrary, Valkenburg-van *et al*. (2009) reported no association between GBS and preterm delivery. However, the association of GBS with PPROM was not specifically identified in their systematic meta-analysis.

In this study, we found that vaginal colonization with *C. albicans* in pregnant women was second most common after GBS. Pregnant women have two-fold increased risk of vulvovaginal candidiasis (VVC), and in many cases, they are asymptomatic (Aguin and Sobel, 2015). Sangaré *et al*. (2018) reported that 22.71% of pregnant women examined in their study had VVC, and amongst them, 40.39% were colonized with *C. albicans*. In another report, as high as 73% of women with VVC were colonized with *C. albicans* (Robert *et al.,* 2015). In our study, 18.3% of the pregnant women had *C. albicans* colonization. Although *C. albicans* has been reported as the major pathogen of VVC during pregnancy, our study showed that PPROM was not associated with the colonization of *C. albicans.* On the other hand, Farr *et al*. (2015) reported that vaginal candidiasis during pregnancy could be a contributing factor to preterm birth and low-birth birth, but a meta-systematic analysis by Roberts *et al.* (2015) revealed that the effect of vaginal candidiasis on preterm birth was inconclusive, as the number of studies were too few.

The risk of vertical transmission of GBS to neonates which lead to sepsis and meningitis have been widely reported, but currently, to the best of our knowledge, there is no existing literature on the rate of vertical transmission of *A. baumannii* from pregnant mothers to their babies. In most reports available, neonatal morbitidy and mortality caused by *A. baumannii* were mainly due to

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Table 1: Isolation of *A. baumannii*, Group B *Streptococcus* and *C. albicans* from vaginal swabs of women with PPROM and normal labor at term and their babies.

Organisms	PPROM M=108, B= 104 n (%)	Normal labor at term M=110, B=111[#] n (%)	Total M=218, B=215 N (%)	P value
Acinetobacter baumannii				
Mother only	8 (7.4)	8 (7.2)	16 (7.3)	0.955
Mother and baby pair	4 (3.8)	2 (1.8)	6 (2.8)	>0.05
Percentage of transfer (%)	50.0	25.0	37.5	
Group B *Streptococcus*				
Mother only	35 (32.4)	12 (10.9)	47 (21.6)	0.000*
Mother and baby pair	12 (11.5)	0	12 (5.6)	0.000*
Percentage of transfer (%)	34.2	0	25.5	
Candida albicans				
Mother only	21(19.4)	19 (17.3)	40 (18.3)	0.2
Mother and baby pair	10 (9.6)	2 (1.8)	12 (5.6)	0.004*
Percentage of transfer (%)	47.6	10.5	30.0	

* Significantly different between PPROM and normal labor at term (p<0.05)
\# Inclusive of a twin
M: mother, B: baby

Table 2: Frequency of co-existent colonization of *A. baumannii*, Group B *Streptococcus* and *C. albicans* in vaginal tract of women with PPROM and normal labor at term.

	PPROM(n) N=108	Normal labor at term (n) N=110	P value
A. baumannii alone	8	8	0.95
GBS alone	35	12	0.00*
C. albicans alone	21	19	0.2
A. baumannii + GBS	1	1	0.53
A. baumannii + *C. albicans*	4	0	0.16
GBS + *C. albicans*	7	3	0.95
A. baumannii + GBS + *C. albicans*	4	1	0.61

* Significantly different between PPROM and normal labor at term (p<0.05), M: mother

nosocomial infection (Huang *et al.*, 2018; Chen *et al.* , 2018). In this study, we observed high probability of vertical transmission of *A. baumannii* from positive mothers to their babies; 50% and 25% in PPROM and NLT respectively. However, the difference was not significant due to the low number of positive cases. This is in contrast to maternal colonisation of GBS whereby babies from mothers with PPROM had a higher risk (34.2%) of contracting GBS from their mothers compared to those who were delivered at term. Our finding showed that none of the babies from NLT mothers were colonized by GBS which is inconsistent with results of meta-systematic analysis by Chan *et al.* (2013) which concluded that babies born at full term from GBS-colonized mothers have 28.6% more chances of acquiring GBS than those babies from non-GBS mothers.

Our study revealed that colonization of *A. baumannii* in the presence of either GBS and/or *C. albicans* did not increase the possibility of PPROM. We postulate that vaginal microbiota, especially Lactobacilli, may out-compete the adherence of GBS and other pathogens to the vaginal epithelial cells as previously demonstrated by Ortiz *et al.* (2014) and *Parolin et al.* (2015). Apart from that, epithelial cells of the vagina were also reported to produce antimicrobial compounds (Yarbrough *et al.*, 2015). Presence of Lactobacilli in the vagina of our subjects was not determined in this study, therefore could not substantiate our postulation.

The limitation of this study was that the sampling of the cases was performed in only one centre. Therefore, this may not be reflective of the true incidence of organisms associated with PPROM.

CONCLUSION

In conclusion, vaginal colonization with *A. baumannii* and *C. albicans* in pregnant women during the third trimester does not predispose them to PPROM, therefore decolonizing is not necessary. However, GBS was associated with PPROM, hence the need for administration of antibiotic prophylaxis during labor as recommended in the guidelines.

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