

## Bacterial vaginosis in pregnancy and early labour using Nugent scoring and the implication on foetal outcome

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### SUMMARY

**Background:** To compare the pattern of vaginal microflora during pregnancy with pattern in early labour using Nugent scoring and determine the effect of these changes on fetal outcome.

**Design:** A prospective longitudinal study.

**Setting and Population:** Pregnant women attending antenatal clinics of Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria between June 2017 and May 2018.

**Methods:** Consenting pregnant women who attended antenatal clinics were recruited. Vaginal secretions were obtained for Nugent scoring during pregnancy and at presentation in labour.

**Main Outcome Measures:** Prevalence of abnormal vaginal flora in pregnancy and early labour, birth outcome, birth weight, gestational age at delivery, APGAR scores, need for neonatal ward admission.

**Results:** Sixty-seven (33.3%) of pregnant women had abnormal flora which was consistent with bacterial vaginosis. At the presentation of these women in labour, 14.4% of them had bacterial vaginosis thus indicating a significant reduction in abnormal vaginal flora in labour compared to the proportion of abnormal flora in antenatal period ( $P < 0.001$ ). There were no significant differences in the fetal outcomes of mothers with bacterial vaginosis when compared with those with normal vaginal flora ( $P$ -value  $> 0.05$ ).

**Conclusions:** Persistence of abnormal vaginal microflora from pregnancy till early labour did not seem to be associated with poorer foetal outcomes when compared with women with normal vaginal microflora in labour. The possibility of persistent infection or re-infection before labour may justify the need for re-evaluation of vaginal smears in the late third trimester to allow for prompt treatment before the onset of labour.

**Keywords:** Bacterial vaginosis, foetal outcome, Lactobacillus, pregnancy, vaginal microflora.

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### INTRODUCTION

The vagina of a normal asymptomatic reproductive-aged woman is populated by several bacterial organisms which include aerobes and obligate anaerobic species.

Of these, the *Lactobacillus* constitutes the main bacterial flora.<sup>1</sup> The vagina and the bacterial communities that reside therein represent a finely balanced mutualistic

association.<sup>2</sup> The vaginal *Lactobacilli* prevent colonization of the vaginal epithelium by other non-indigenous microorganisms by contributing to the maintenance of a low vaginal pH(4.5) through the production of lactic acid.<sup>3</sup> *Lactobacilli* also play a key role in maintaining the health of the vagina by generating hydrogen peroxide which has a cleaning and anti-infection property.<sup>4,5</sup>

It has been shown that the vaginal microflora is more stable in pregnant women than non-pregnant women.<sup>1</sup> This is due to the high levels of circulating estrogens during pregnancy. In a considerable number of women, vaginal lactobacilli fail to retain dominance. This usually results in vaginal flora's symbiotic relationship shifting to one in which there is an overgrowth of an anaerobic species including *Gardnerella vaginalis*, *Ureaplasma-urealyticum*, *Mobiluncus* species, *Mycoplasma hominis*, and *Prevotella* species, a condition referred to as bacterial vaginosis. Loss of the indigenous *Lactobacilli* strongly predisposes to ascending genital tract infection, which in pregnancy is a major cause of chorioamnionitis, amniotic fluid infection and preterm birth.<sup>6-8</sup> Studies have also shown that the depletion of vaginal *Lactobacillus* microflora predisposes women to the acquisition of sexually transmitted infectious diseases caused by organisms such as *Neisseria gonorrhoea*, *Chlamydia* species and Human Immunodeficiency Virus.<sup>9,10</sup>

The presence of different *Lactobacillus* species within the normal vaginal microflora is a major determinant of the stability of this microflora in pregnancy.<sup>11</sup> There has been no study conducted in our environment to investigate the changes in vaginal microflora during an ongoing pregnancy. Findings from such studies may justify the need to apply strategies aimed at maintaining the normal vaginal microflora during pregnancy. This may include the introduction of probiotics that maintain normal vaginal *Lactobacilli* population in pregnant women and offering treatment to those with abnormal flora. This may ultimately lead to more favourable fetal outcomes by preventing ascending genital tract infections. An assessment of vaginal microflora in pregnancy and early labour will identify the changes that occur in vaginal microflora during pregnancy and determine the effect of these changes on fetal outcome. It will also help to evaluate the effectiveness of empirical treatment of abnormal microflora. The aim of this study is to compare the pattern of vaginal microflora during pregnancy with pattern in early labour using Nugent's scoring.

## METHODS

This prospective longitudinal study was conducted at the antenatal clinics and labour ward of Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Ogun State Nigeria.

This hospital is one of the two teaching hospitals for referral from clinics, maternity homes and hospitals in Ogun State, Nigeria. Attendance in the hospital is unrestricted and hence the patients are of mixed socioeconomic and religious background. Obstetric services are provided for both high and low-risk pregnant women by nurses and midwives, resident doctors undergoing specialist obstetrics and gynaecology training and consultant obstetricians and gynaecologists.

The study population consisted of pregnant women who received antenatal care in OOUTH. The calculated minimum sample size was 144. The addition of 10% attrition increased the sample size to 158. However, 408 pregnant women consented to participate in the study and were consecutively recruited from 1<sup>st</sup> of June 2017 to 31<sup>st</sup> of May 2018. The gestational age at recruitment was from 26weeks to 32weeks gestation. Women with history of vaginal discharge, bleeding per vagina or those who took antimicrobials within the preceding two weeks were excluded from the study. The pregnant women were given adequate information on the study and those who agreed to participate in the study signed a written consent form.

## Sample Collection and Processing

With the aid of a sterile disposable Cusco's speculum, vaginal secretion samples were taken from the posterior fornix using three sterile swab sticks. For clinical characteristic, the vaginal secretion was examined in terms of colour, homogeneity and consistency. One of the three swab stick was examined for pH of the secretion using the standard pH paper and pH was recorded. Two drops of 10% potassium hydroxide (KOH) was added to the swab stick and result of the whiff test recorded. The other two swab specimens were appropriately labelled and immediately sent to the OOUTH medical microbiology laboratory for immediate processing. At the laboratory, one swab stick was smeared on a plain glass slide and allowed to air dry, heat-fixed, Gram stained and examined under a light microscope. The scores were assigned according to Nugent's criteria<sup>12</sup>. The Nugent's criteria are based on the relative proportions of large Gram-positive rods, small Gram-negative/Gram variable rods and curved Gram-variable rods i.e. *Lactobacillus*, *Bacteroides/Gardnerella*, and *Mobiluncus* morphotypes respectively. Grading is done on a scale of 1-4 (1+ is < 1 cell per field, 2+ is 1-5 cells per field, 3+ is 6-30 cells per field, and 4+ is >30 cells per field). In this system, *Lactobacillus* and *Bacteroides/Gardnerella* are given scores between 0-4 but *Mobiluncus* is only graded from 0-2.

Total scores are then calculated and used as follows: 0-3 (normal), 4-6 (intermediate), and 7-10 (abnormal microflora consistent with bacterial vaginosis).

The other swab stick was streaked onto Saboraud-Dextrose Agar (SDA), blood Agar (BA) and Mac Conkey Agar (MCA). The inoculated plate was incubated aerobically at 37°C for 24 hours and subsequently for another 24 hours if no growth. Isolates from SDA were identified to species level using chrome agar candida (Hardy Diagnostics, Santa Maria, CA) and isolates from BA and MCA were identified and analysed using colonial morphology and biochemical methods. Women with bacterial vaginosis were treated according to departmental protocol with oral metronidazole 400mg twice daily for seven days. They were also given a vaginal pessary containing metronidazole, clotrimazole and *Lactobacillus* spores (Klovinal®) to assist in maintaining the normal vaginal ecosystem and also treat *Candida* infection which is common in women with abnormal vaginal microflora. Those with positive bacterial culture received antibiotics based on their antibacterial sensitivity pattern. Their partners were also treated with the same antibiotics.

The pregnant women were then followed up until they presented in labour when a repeat sample collection and processing was done prior to rupture of membranes. Fetal outcome measures assessed were the birth outcome, gestational age at delivery, birth weight, Apgar scores at first and fifth minutes of life and need for neonatal ward admission. Low birth weight was defined as a birth weight less than 2.5kg.

**Ethical considerations**

The ethical approval for the study was obtained from the Health Research Ethics Committee of Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria with reference number OOUTH/HREC/59/2016.

**Data management and analysis**

Data were analysed using Statistical Package for Social Science (SPSS) windows version 21. The socio-demographic characteristics of the participants were presented using a frequency table. Continuous variables were summarised using descriptive statistics such as mean and standard deviation at 95% confidence interval. Categorical variables were summarized by frequencies and percentages. The influence of vaginal microflora patterns in labour on foetal outcome was assessed using chi-square test. A *P*-value of less than 0.05 was considered significant.

**RESULTS**

A total of 408 consenting pregnant women were recruited for the study, however, 201 women (49.3%) had their deliveries in the hospital and thus had complete labour data. The age range of the women was from 17 to 42 years with a mean age of 29.35 ± 5.20 years.

The majority (49.5%) of the women were from the age group 20-29 years. The mean parity was 1.07 ± 1.08. One hundred and sixty-five women (40.4%) were nullipara and the majority (60.3%) of the women had tertiary education (Table 1).

**Table 1** Socio-demographic characteristics of participants

Variable	N (%)	Mean ± SD
<b>Maternal Age (years)</b>		29.35±5.2
≤ 19	07 (1.7)	
20 – 29	202 (49.5)	
30 – 39	190 (46.6)	
≥ 40	09 (2.2)	
<b>Parity</b>		1.07±1.08
Para 0	165 (40.4)	
Para 1 - 2	199 (48.8)	
Para 3 - 4	42 (10.3)	
Para ≥ 5	02 (0.5)	
<b>Occupation</b>		
Unskilled	14 (3.4)	
Semi-skilled /Skilled	329 (80.6)	
Professional	65 (15.9)	
<b>Educational level</b>		
≤ Primary	19 (4.7)	
Secondary	143 (35.0)	
Tertiary	246 (60.3)	
<b>Type of marriage</b>		
Monogamy	382 (93.6)	
Polygamy	26 (6.4)	
<b>Tribe</b>		
Yoruba	292 (71.6)	
Igbo	57 (14.0)	
Hausa	03 (0.7)	
Others	56 (13.7)	
<b>Religion</b>		
Christianity	308 (75.5)	
Islam	98 (24.0)	
Others	02 (0.5)	

Table 2 shows the characteristics of vaginal secretions during pregnancy and early labour among women that delivered at the study centre.

Ten per cent of the women tested positive to whiff at the presentation in early labour. There was a statistically significant difference in the whiff test results between antenatal women and women in early labour (*P*=0.001). The vaginal pH was ≥5 in the majority of the women both in pregnancy and early labour with 98% and 96.5% prevalence respectively. The mean pH of the vaginal secretion during pregnancy was 5.93±0.82 while it was 6.34±0.88 in early labour. There was no significant difference in vaginal pH during pregnancy and during labour (*P* = 0.359).

Nugent's classification of vaginal secretions shows a statistically significant reduction in the proportion of abnormal vaginal flora in labour compared to the proportion of abnormal flora in the antenatal period ( $P < 0.001$ ).

**Table 2** Comparison of the characteristics of vaginal secretions in pregnancy and at delivery

Vaginal secretions	Antenatal N (%)	Early Labor N (%)	X <sup>2</sup>	P value
<b>Whiff test</b>			11.47	0.001*
Positive	45 (22.4)	20 (10.0)		
Negative	156 (77.6)	181 (90.0)		
<b>Vaginal pH</b>			0.84	0.359
< 4	04 (2.0)	07 (3.5)		
≥ 5	197 (98.0)	194 (96.5)		
<b>Nugent score</b>			38.25	0.000*
0 – 3	11 (5.5)	48 (23.9)		
4 – 6	123 (61.2)	124 (61.7)		
7 – 10	67 (33.3)	29 (14.4)		

\*P-value < 0.05

Out of the 201 women that delivered in our hospital, 193 (96%) had live-births. Twenty nine (14.4%) had preterm delivery while majority (85.6%) delivered at term. The

mean gestational age at delivery was  $38.52 \pm 2.20$  weeks. The majority (89.1%) of the women delivered babies with normal birthweights (2.50 – 3.99kg). The mean birth weight of the babies was  $3.12 \pm 0.50$ kg. The mean Apgar scores at first and fifth minutes of life were  $7.39 \pm 1.21$  and  $8.50 \pm 1.20$  respectively. Only 8.5% of all the newborns required neonatal admission. A majority (80.6%) of the babies were delivered via the vaginal route.

Table 3 shows the association between Nugent scoring pattern in labour and foetal outcome. One hundred and ninety-three women delivered live babies representing 96% of 201 women. Twenty-nine (15%) of the mothers had abnormal vaginal flora consistent with bacterial vaginosis. No stillbirth was recorded in women with abnormal flora in this study. Only three (10.3%) of the mothers with preterm deliveries had abnormal vaginal flora.

Two (6.9%) of the 29 women with abnormal vaginal flora delivered low birthweight babies. Majority of the women with abnormal vaginal flora had babies with good Apgar scores at first and fifth minutes of life (72.4% and 96.6% respectively). Most of the babies (93.1%) from mothers with abnormal vaginal flora did not require neonatal admission. There was no statistically significant association between the Nugent scoring pattern and foetal outcomes of the mothers (P-value > 0.05).

**Table 3** The pattern of Nugent scoring in labour on foetal outcome

Fetal outcome	Labor Nugent score			Mean±SD	X <sup>2</sup>	P value
	Normal N (%)	Intermediate N (%)	Abnormal N (%)			
<b>Birth outcome</b>						
Livebirth	44 (91.7)	120 (96.8)	29 (100)			
Stillbirth	04 (8.3)	04 (3.2)	0 (0)		3.77	0.152
<b>GA at delivery (weeks)</b>						
< 37	10 (20.8)	16 (12.9)	03 (10.3)	38.53±2.20	2.22	0.330
≥ 37	38 (79.2)	108 (87.1)	26 (89.7)			
<b>Birthweight (kg)</b>						
LBW	05 (10.4)	11 (8.9)	02 (6.9)			
NBW	43 (89.6)	113 (91.1)	27 (93.1)	3.12±0.50	0.28	0.870
<b>Apgar score at 1 min</b>						
< 7	05 (10.4)	14 (11.3)	08 (27.6)			
≥ 7	43 (89.6)	110 (88.7)	21 (72.4)	7.39±1.21	5.86	0.053
<b>Apgar score at 5 mins</b>						
< 7	02 (4.2)	09 (7.3)	01 (3.4)			
≥ 7	46 (95.8)	115 (92.7)	28 (96.6)	8.50±1.20	0.97	0.615
<b>Need for Neonatal Ward admission</b>						
Yes	03 (6.3)	12 (9.7)	02 (6.9)			
No	45 (93.7)	112 (90.3)	27 (93.1)		0.72	0.632

Nugent's score: Normal flora (0-3); intermediate flora (4-6); abnormal flora (7-10), SD = standard deviation, GA = gestational age, LBW = low birthweight, NBW = normal birthweight, NNW = neonatal ward

*Candida* species were isolated in 42.3% of women during pregnancy compared to 45.8% of women in early labour. There was no significant difference in the *Candida* isolates during pregnancy and labour. *Candida albicans* was the commonest candida species accounting for 64.7% and 75% of the *Candida* growth during pregnancy and labour respectively. The most predominant bacterial isolate in pregnancy and during labour was *Staphylococcus aureus* accounting for 61.9% and 72.7% respectively. While *Escherichia coli* and *Klebsiella species* accounted for 4% each and being least predominant bacterial isolates during pregnancy, there was no *Escherichia coli* isolate during labour. *Klebsiella species* was the least predominant bacterial organism in labour (Table 4).

## DISCUSSION

The vagina of a reproductive age woman is populated by several bacterial organisms which include aerobes and obligate anaerobic species, and of these, the presence of Lactobacillus species is a major determinant of normal vaginal microflora. This study assessed the pattern of vaginal microflora during the antenatal period and early labour and our findings suggest that there is a statistically significant reduction in the prevalence of abnormal vaginal flora in labour when compared to the proportion of abnormal flora during the antenatal period. There also appears to be no significant effect of abnormal vaginal flora on foetal outcome.

Out of the 408 women recruited for the study, 201 (49.3%) delivered in the hospital and thus had complete data. A previous study at this centre, reported 47.6% of antenatal attendees came back to deliver in the hospital.<sup>13</sup> Several methods have been proposed to characterize the vaginal microflora including Amsel's criteria (adherent homogenous vaginal discharge, elevated vaginal pH, presence of clue cells and a positive whiff test), Nugent's classification, culture method and molecular methods based on the analysis of the 16S rRNA gene fragment.<sup>14,15</sup> Nugent's classification for the diagnosis of bacterial vaginosis has been shown to be more reliable than other techniques.<sup>14,16</sup> In our study 45 (22.4%) of the 201 pregnant women with complete data had a positive whiff test at recruitment (during antenatal visits) whereas only a tenth of them had a positive whiff test at presentation in labour. This showed a significant reduction in proportion of pregnant women with a positive whiff test. Majority of the women during pregnancy and at presentation in labour had elevated vaginal pH, however, there was no significant difference in the vaginal pH in antenatal and early intrapartum periods ( $p=0.359$ ).

**Table 4** Vaginal microbial isolates in pregnancy and labour

Microbial isolates	Antenatal N (%)	Early labour N (%)	X <sup>2</sup>	P value
<b>Candida growth</b>			0.495	0.482
Positive	85 (42.3)	92 (45.8)		
Negative	116 (57.7)	109 (54.2)		
<b>Bacterial organisms</b>				
<i>Staph aureus</i>	78 (61.9)	32 (72.7)		
<i>Streptococcus sp.</i>	38 (30.2)	11 (25.0)		
<i>E. coli</i>	05 (4.0)	0 (0.0)		
<i>Klebsiella sp.</i>	05 (4.0)	01 (2.3)		

There is evidence to suggest that positive whiff test is associated with a high specificity (85.7%) and accuracy rate (74.4%) within the Amsel's criteria for clinical diagnosis of bacterial vaginosis.<sup>17</sup> However, studies have consistently shown that vaginal pH was a relatively poor predictor of bacterial vaginosis.<sup>18,19</sup>

This trend was also observed in our study wherein the Nugent's scoring system indicated that only a third of the antenatal women had abnormal vaginal flora (Nugent's score  $\geq 7$ ) whereas using vaginal pH, 98% of antenatal women will be presumed to have abnormal vaginal flora defined as pH  $\geq 5$ .

The prevalence of bacterial vaginosis among pregnant women was 33.3%. Other studies have indicated prevalence rates ranging from 17.3% to 60%.<sup>16,20,21</sup> However, the prevalence of bacterial vaginosis among women presenting in labour was 14.4% in our study. This showed a decline from the proportion of abnormal vaginal flora during the antenatal period which may be a reflection of the efficacy of the treatment offered during pregnancy. Another reason which may be adduced to this lower prevalence may be the fact that advancing gestational age with a consequent increased level of pregnancy hormones including estrogen which is responsible for the accumulation of glycogen on the vaginal epithelium.

The glycogen serves as a substrate for Lactobacilli species for the production of lactic acid which in turn maintain the stability of the vaginal microflora and thereby reduce the risk of overgrowth of anaerobic organisms. Apart from increasing estrogen levels with advancing gestational age, reduced coital frequency in late pregnancy has also been found to be associated with less incidence of distortion in vaginal microflora.<sup>22</sup> In our study, women with abnormal vaginal flora during pregnancy, and their partners were treated.

Despite this, 14.4% of these women still had abnormal vaginal microflora consistent with bacterial vaginosis based on the Nugent's classification in the early phase of their labour. Although this value is lower than the reported prevalence in pregnancy, it may also indicate re-infection of the vaginal ecosystem. The possibility of persistent infection or re-infection before labour may justify the need for re-evaluation of vaginal smears in the late third trimester of the larger population of pregnant women so as to allow for prompt treatment before the onset of labour.

It should, however, be noted that this was not associated with poor foetal outcomes. All the women with the abnormal vaginal flora delivered live-births. Furthermore, there was no significant difference in the proportion of adverse foetal outcome in terms of preterm birth, low birth weight or neonatal ward admission between those with normal and abnormal vaginal flora.

These observations are similar to report from an Indian study.<sup>23</sup> However, Afolabi et al<sup>20</sup> in Lagos, Nigeria, reported a higher risk of preterm delivery and low birth-weight babies. This may be due to their higher prevalence (26%) of bacterial vaginosis compared to ours which was 14.4%. The higher prevalence recorded in their study may also be because analyses were done before labour against the background of the declining prevalence of bacterial vaginosis with advancing gestational age. In addition to standard oral therapy for bacterial vaginosis, our clients also received a combination of metronidazole, clotrimazole and lactobacillus spores boosted vaginal pessaries (Klovinal®) as a local treatment to re-establish the dominance of Lactobacillus species in the vagina before the onset of labour. This strategy might have contributed to the good perinatal outcome in this study.

Apart from the vaginal flora assessment with Nugent scoring, additional microbial organisms were also isolated and these included *Candida* species and some bacterial organisms. Contrary to the findings relating to abnormal vaginal flora (by Nugent's scoring), there was no statistically significant difference in the distribution of the *Candida* species and bacterial isolates between antenatal and early labour. This was despite the fact that both infected women and their partners were treated during the antenatal period. This finding may also reflect treatment failure or re-infection. There is need for further studies to clarify this. The prevalence of asymptomatic candidiasis was 42.3% and 45.8% in pregnancy and early labour, respectively. A similar finding was reported from a previous study in this hospital where a prevalence of 44.8% was reported among asymptomatic pregnant women.<sup>24</sup>

Vaginal candidiasis has been noted to be common in pregnancy but there seems to be no evidence of untoward effects on the perinatal outcome.<sup>25</sup>

## CONCLUSION

A third of pregnant women have abnormal vaginal microflora in pregnancy. Despite empirical treatment, about one in seven women retained abnormal vaginal microflora in early labour. Persistence of abnormal vaginal microflora till early labour in our study did not seem to be associated with poorer foetal outcomes in terms of preterm birth or need for neonatal ward admission when compared with women with normal vaginal microflora in labour..

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