

1 PATTERN OF MATERNAL GROUP B STREPTOCOCCUS (GBS) COLONIZATION IN RELATION TO
2 CD4 COUNT AMONG HIV POSITIVE WOMEN IN JOS

3 **Original Research Article**

4 **ABSTRACT:**

5 **Aim**

6 The aim of this study was to determine the prevalence of GBS colonization among HIV positive and HIV
7 negative pregnant women in relation to CD4 cell counts

8 **Materials and Methodology**

9 This was a hospital based descriptive cross-sectional study of 200 pregnant women (100 HIV positive and
10 100 HIV negative) and 100 non-pregnant women (50 HIV positive and 50 HIV negative) obtaining health
11 care at the Jos University Teaching Hospital between July 2017 and November 2017. Systematic sampling
12 technique and written informed consent were used in recruiting subjects for this study. High vaginal and
13 anorectal swabs were collected from each subject after filling a structured questionnaire. CD4 cell count
14 was also done for all the HIV positive patients at Aids Prevention Initiative in Nigeria (APIN) of Jos
15 University Teaching Hospital (JUTH). The results from the laboratory analysis of the specimens were
16 computed using SPSS version 21.

17 **Results**

18 A colonization rate of 7.3% was observed in HIV positive patients compare to 5.3% in HIV negative. The
19 different in colonization rate between the two groups was not statistically significant ($\chi^2 = 0.507$; $P = 0.477$)
20 (table 1). In pregnant women living with HIV, colonization rate was 8.0% compare to 5.0% observed in non-
21 pregnant women living with HIV. This however, was not statistically significant (Table 2) ($\chi^2 = 0.013$; $P =$

22 0.908). HIV positive subjects with low CD4 counts (<200cells/ μ l) were observed to have high colonization
23 rate (20.0%) than patients with high CD4 counts (\geq 500 cells/ μ l). Those with CD4 counts between 200-499
24 cells/ μ l had 8.1% colonization rate. These findings, though not statistically significant (table 4) ($\chi^2 = 1.3814$;
25 $P = 0.2399$), the increased colonization rate in low CD4 cell counts may be due to inability of the patient to
26 mount immune response against the organism.

27 Conclusion

28 There was no statistically significant difference in GBS colonization among HIV positive patients. A higher
29 colonization rate was observed in HIV patients among the age group 21-25 years; age was not
30 significantly risk factor for GBS colonization in HIV patients. CD4 cell counts seem not to play any
31 significant role in GBS colonization rate. Although, it was observed to be higher in patients with low CD4
32 cell counts; the difference was not statistically significant.

33 INTRODUCTION

34 *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) has been documented as the leading infectious
35 agent responsible for neonatal morbidity and mortality [1]. The primary risk factor for newborn disease is
36 colonization of the maternal gastrointestinal tract and vagina by GBS [2]. The newborn acquired the
37 infection by vertical transmission of GBS from a vagina-colonized mother upon rupture of membrane or
38 after the onset of labour. This can lead to life-threatening infections such as sepsis and meningitis [3].

39 Maternal vaginal colonization by GBS in late pregnancy or at delivery is the main factor associated with
40 both early onset neonatal diseases (EOD) and late onset neonatal diseases (LOD) [4]. GBS colonizes the
41 human gastrointestinal and genital tract of 20-30% of healthy humans. The colonization of the vagina can
42 be transient, intermittent or chronic and serve as potential source of infection to the newborn [5,6].

43 The colonization in pregnant women may remain asymptomatic or may be associated with spontaneous
44 abortion [7], chorioamnionitis [8], premature rupture of membrane which may result in serious neonatal and
45 maternal morbidity and mortality [9,10]. This link has motivated the recommendation of universal antenatal
46 screening of pregnant women for GBS at 35-37 weeks of gestation and the administration of intrapartum
47 antibiotic prophylaxis (IAP) in patients with colonization result [11]. This strategy has been associated with a
48 significantly decreased incidence of EOD but has limited impact on the incidence of LOD [12].

49 Many factors have been established to influence the vaginal colonization by GBS [13]. However, conflicting
50 reports have emerged as to the role of HIV infection as risk factor for GBS colonization. While Cutland and
51 his colleagues in 2012 reported that GBS colonization is lower in HIV infected than HIV uninfected women
52 [14], Gray et al 2011 [15] and Shah et al 2011 [16] have earlier documented in separate studies that HIV
53 infection is not independently associated with GBS. Nevertheless, the proportion of women colonized with
54 GBS is significantly higher in HIV-infected women with a CD4 cell count higher than 500cells/mm³ when
55 compared to women with a CD4 cell count lower than 200 cells/mm³ [14,15]. It has also being
56 demonstrated that GBS colonization is significantly higher in HIV infected women with higher CD4 cell
57 counts than HIV negative women [15]. The increased colonization rate in HIV-positive women with high
58 CD4 cell count might be biased by the presence of other risk factors for GBS colonization like diabetes or
59 obesity [4]. On the other hand, HIV-infected women with low CD4 cell count are known to have increased
60 prevalence of bacterial vaginosis that could compete with GBS and are more likely to take cotrimoxazole
61 prophylaxis resulting in lower GBS carriage rates [17].

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63

64 MATERIALS AND METHODS:

65 Study Area

66 The study was carried out in Jos University Teaching Hospital (JUTH). JUTH is located in Jos the Plateau
67 State capital. The hospital is a tertiary health institution with a 600 beds capacity serving Plateau State and
68 majority of the states in the North-central and part of North-east geopolitical zones of Nigeria. JUTH is also
69 a centre for AIDS Prevention Initiative in Nigeria (APIN) that cater for most people leaving with HIV (PLHIV)
70 from within and the bordering states. The main occupation of the people is farming with majority of them in
71 the city being civil servants and businessmen and women.

72 Study Population

73 The study population included HIV positive and HIV negative women attending antenatal clinic at the Jos
74 University Teaching Hospital between July 2017 and November 2017.

75 Study Design

76 The study was a hospital based descriptive, cross-sectional study that recruited 300 consenting pregnant
77 and non-pregnant women attending antenatal and gynaecology clinics at the Jos University teaching
78 Hospital.

79 Ethical Consideration

80 This study was approved by the research ethical committee of Jos University Teaching Hospital with
81 reference number JUTH/DCS/ADM/127/XIX/6583. Written informed consents were also signed by all
82 subjects before enrollment in the study.

83

84 Sample Collection

85 Anorectal and vaginal swabs were carefully and aseptically collected from 150 HIV positive and 150 HIV
86 negative women using sterile swab sticks by the attending physicians after given them appropriate
87 instructions on how the sample should be collected (CDC, 2010).

88 Specimen Transport

89 The collected specimens were immediately inoculated into a selective enrichment broth, Todd - Hewitt
90 broth (Oxoid LTD) supplemented with gentamycin (8µg/ml), nalidixic acid (15µg/ml) and 5% sheep blood to
91 increase the recovery rate of GBS [18,19]. These were transported to the laboratory within three hours of
92 inoculation.

93 Culture and Incubation

94 The tubes of inoculated Todd-Hewitt broth were incubated aerobically at 37°C for 18 to 24 hours. After an
95 overnight incubation, the broths were subcultured onto 10% sheep blood agar and chromatic Strepto B
96 agar (Liofilchem, Italy), a selective medium for GBS.

97 The inoculated 10% sheep blood agar plates were incubated aerobically in 5-10% CO₂ (candle extinction
98 jar) at 37°C for 18 to 24 hours, while the inoculated chromatic Strepto B agar plates were incubated
99 aerobically at 37°C for 18 to 24 hours [20]. The *Streptococcus agalactiae* control strain was also inoculated
100 onto 10% sheep blood agar and chromatic Strepto B agar and incubated as stated above respectively.

101 Identification of GBS Isolates

102 GBS isolates were identified by their beta haemolytic pattern on 5% sheep blood agar and blue-green
103 colour on chromatic Strepto B agar. The isolates were further subjected to Gram staining, catalase test,
104 and Serogrouping using streptococcal grouping kit (DR0585A OXOID) from Oxoid.

105

106 Data Analysis

107 The data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS)
108 version 21 (IBM SPSS Inc, USA). Proportions were compared using Chi-square with confidence limit (p-
109 value) of < 0.05 considered significant.

110

111 RESULTS

112 Of the 150 HIV positive participants, 100 were pregnant women while 50 were non-pregnant women. The
113 overall GBS colonization among the HIV positive women was 7.3%. The colonization rate of 8.0% was
114 observed in pregnant women with HIV compare to 5.0% non-pregnant women with HIV (Table 1).

115 In addition, GBS colonization did not appear to be influenced by maternal age in HIV infected women. The
116 study revealed a higher colonization rate of 15.4% among the age group 21-25 years followed by age
117 group 31-35 years. Result from age group 16-20 years was negative as no GBS was isolated among this
118 age group (Table 3) ($\chi^2 = 1.3814$; $P = .24$).

119 The correlation between GBS colonization and CD4 cell counts was also analyzed. It was observed that
120 CD4 cell counts were not independently associated with GBS colonization. The result showed that women
121 with CD4 cell counts of <200cells/ μ l were colonized in 20.0%. Those with CD4 counts between 200-499
122 cells/ μ l were positive in 8.1%. Based on our findings, the colonization rate was lower in those with CD4
123 counts \geq 500 cells/ μ l as only 5.1% were positive for GBS (Table 4). This study has demonstrated that
124 though colonization is higher in HIV positive women with low CD4 cell count, the difference is not
125 statistically significant ($\chi^2 = 1.702$; $P = .43$).

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128 Table 1
 129 Group B Streptococcal carriage rates among HIV positive and HIV negative women in Jos University
 130 Teaching Hospital

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HIV Status	No. Tested	No. Positive	Percentage positive
Positive	150	11	7.3%
Negative	150	8	5.3%
Total	300	19	6.3%

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133 $\chi^2 = 0.506$ $P = .48$ $df = 1$

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138 Table 2
 139 Group B Streptococcal carriage rates among HIV positive and HIV negative women in Jos University
 140 Teaching Hospital

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Pregnant women	Non-Pregnant women
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HIV Status	No. Tested	No. Positive	No. Tested	No. Positive (%)
Positive	100	8 (8%)	50	3 (6%)
Negative	100	5 (5%)	50	3 (6%)
Total	200	13 (6.5%)	100	6 (6.0%)

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143 $\chi^2 = 0.013$ P = .91 df = 1

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153 Table 3

154 Carriage of *Streptococcus agalactiae* among HIV positive women according to maternal age in Jos

155 University teaching Hospital

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Age Group (years)	HIV Positive	
	No. Tested	No. Positive (%)
16-20	1	0(0.0)
21-25	13	2(15.4)
26-30	33	1(3.0)
31-35	40	4(10.0)
36-40	41	3(7.3)
≥40	22	1(4.5)
Total	150	11(7.3)

157

158 $\chi^2 = 1.3814$ P = .24 df = 5

159

160 Table 4

161 *Streptococcus agalactiae* colonization in relation to CD4 cells count in HIV positive women in Jos University

162 Teaching Hospital

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CD4 Cell Counts (cells/ μ l)	No. Tested	No. Positive	% Positive
<200	5	1	20.0
200-499	86	7	8.1
≥ 500	59	3	5.1
Total	150	11	7.3

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166 $\chi^2 = 1.702$ P = .43 df = 2

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173 DISCUSSION

174 This study recruited 150 HIV positive and 150 HIV negative women. The result has shown that HIV
175 infection was not independently associated with GBS colonization among women. This is in contrast to
176 report by Lekala and his colleagues in South Africa that HIV infection is significantly associated with GBS
177 colonization than in HIV negative women [21]. We also observed that HIV infected pregnant women had a
178 carriage rate of 8.0%, though higher than the rate of 5.0% in HIV negative pregnant women, it was not
179 statistically significant. This means that HIV infection is not independently associated with GBS colonization
180 among pregnant women. This finding is comparable with the report in Malawi where the correlation of GBS
181 and HIV was analyzed but no association was found between HIV seropositive and GBS colonization in
182 pregnancy [15].

183 This study also revealed that the age group 21-25 years among HIV infected women is associated with
184 higher GBS colonization rate followed by the age group 31-35 years, though; this association was not
185 statistically significant. This is a sexually active age group and it has been stated that vaginal colonization
186 by GBS is associated with sexual intercourse [22,23]. However, this research did not correlate the
187 importance of sexual intercourse in this age group to GBS colonization. Ezeonu and his colleagues in 2012
188 in Enugu state of Nigeria documented that GBS carriage rate is higher in women between the age group
189 21-25 years [24]. This finding was similar to the report of Dzewela *et al* (2005) and Lekala *et al* (2015) but in
190 contrast with some other research findings that reported increase in GBS colonization with advanced
191 maternal age although no reason was stated [15,25].

192 It was also observed that the rate was higher in HIV positive patients with low CD4 counts and less
193 frequent in those with CD4 counts ≥ 500 cells/ μ l though the difference was not statistically significant. This
194 is contrary to the report by Gray *et al* (2011) and Shah *et al* (2011) that GBS carriage rate significantly
195 increased at higher CD4 counts [15,16].

196 CONCLUSION

197 In conclusion, this research has demonstrated that HIV infection is not a risk factor associated with GBS
198 colonization. Even though colonization rate was more in HIV positives compare to HIV negative patients, it
199 was not statistically significant. Also, this study was able to establish that CD4 cells count is not significantly
200 associated with GBS colonization. The rate was higher in patients with low CD4 cell counts with no
201 statistical difference. In addition, colonization rate was observed to be higher in HIV patients among the age
202 group 21-25 years; age was not found to be a contributor to GBS carriage rate.

203 Consent for publication

204 All the authors reviewed and gave their consent for this article to be submitted for publication.

205 Ethical approval

206 As per international standard or university standard, written approval of ethics committee has been
207 collected and preserved by the authors.

208 Competing of interest

209 There are no conflicts of interests among the authors.

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