

CORRELATION OF H₂O₂ PRODUCTION OF LACTOBACILLUS SPECIES WITH NUGENT SCORES OF YOUNG NIGERIAN WOMEN

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ABSTRACT

Aim: To correlate H₂O₂ production of Lactobacilli specie with the Nugent score of young Nigerian women in order to assess their vaginal health.

Study design: Titration method

Place and Duration of Study: Department of Biochemistry and Department of Medical Microbiology, College of Medicine of the University of Lagos, between May and august 2009.

Methodology: 97 isolates of Lactobacilli from both women with and without Bacterial vaginosis (BV) was used for the study. The Lactobacilli isolates were categorized into facultative anaerobes and strict anaerobes. Hydrogen peroxide was detected and measured by titration using dilute sulphuric acid and reaction stopped with potassium permanganate.

Results: Out of 97 isolates studied, 76 (78%) were facultative anaerobes, while 21 (22%) were strict anaerobes. The facultative anaerobes were obtained from 11 of 15 women with BV and 65 of 82 women without BV. Forty nine (50.51%) of the 97 isolates produced H₂O₂. Forty four of the H₂O₂ producers were from women without BV while five were from women with BV. Majority of the strains obtained from women

with BV were non-hydrogen peroxide producing. Rates of H₂O₂ production by Nugent score were 70%, 43% and 33% in negative, intermediate and BV Nugent scores respectively. There was no significant difference between the mean concentrations of H₂O₂ production in the various Nugent scores.

Conclusion: The overall rate of hydrogen peroxide production was low. While the rates of hydrogen peroxide production correlated with Nugent scores, being highest in negative Nugent scores and lowest with BV scores, the concentration of hydrogen peroxide produced had no association with Nugent scores. Nigerian women may have a relatively high susceptibility rate to vaginal infections.

Keywords: *Lactobacillus*, *Nugent score*, *Bacterial vaginosis*, *hydrogen peroxide production*

INTRODUCTION

Lactobacilli are the predominant flora in the vagina of premenopausal women. They occur as either facultative anaerobe, which grow more abundantly in the presence of carbon dioxide or as strict anaerobes¹. Their presence is believed to regulate the growth of other vaginal flora. This is because they produce compounds such as lactic acid and hydrogen peroxide which inhibit their growth. The presence of lactic acid helps to reduce the vaginal pH, making it acidic and this selects for aciduric organisms, while the production of hydrogen peroxide inhibits the growth of organisms that lack enzymes such as peroxidase and catalase².

The absence or reduction of hydrogen peroxide producing lactobacilli in the vagina could therefore lead to colonization of the vagina by other microbial flora, which are normally present in small numbers¹⁻⁴. Such organisms include *Gardnerella vaginalis* and some anaerobes, the overgrowth of which is associated with biochemical changes including increased concentration of diamines, polyamines, organic acids and enzymes such as mucinases, sialidases and collagenases in vaginal fluid.³⁻⁴ These biochemical end products cause a further increase in pH and a vaginal discharge with an unpleasant odour⁴. The condition, which is called bacterial vaginosis (BV) is asymptomatic in many women, but has also been associated with a number of obstetric and gynaecologic complications including endometritis, pelvic inflammatory disease, miscarriages, premature rupture of membranes and preterm birth^{5,6}. The diagnosis of BV is therefore important whether it is symptomatic or not. This has led to the validation and acceptance of Nugent score especially for the asymptomatic woman.

The Nugent scoring system (zero to 10) is a weighted combination of bacterial morphotypes present in the vagina depending on the presence or absence of BV. The morphotypes assessed include *Lactobacillus* (large gram-positive rods), *Gardnerella vaginalis* and Bacteroides species (small gram-variable or gram-negative rods), and Mobiluncus species (curved gram-variable rods). The total score is the sum of the weighted quantity of the three bacterial morphotypes. Scores 0 - 3, obtained when the flora is predominantly lactobacillus is accepted as negative for BV while scores 7 -10 which relatively devoid of lactobacillus and may show the Mobiluncus spp morphotype, is accepted as positive for BV⁷ Some studies have shown that not all strains of lactobacillus produce hydrogen peroxide and their presence does not necessarily indicate vaginal protection^{6,8}. This may explain why at Nugent scores 7 and 8, which are diagnostic of BV may also be associated with the presence of Lactobacillus on microscopy⁸. In order to assess the vaginal health of women and speculate susceptibility to reproductive infections, this study was done to detect hydrogen peroxide production in some lactobacillus species previously isolated, quantify the produced H₂O₂ and to correlate the amounts produced with Nugent score 0 to 10..

2. METHODS

Between May and August 2009, hydrogen peroxide production and quantification was carried out by titration method at the Department of Biochemistry, College of Medicine of the University of Lagos, Nigeria, on 97 isolates of lactobacillus obtained from the Medical Microbiology Department of the same institution. It was approved by the Health Research Ethics Committee of the Lagos University Teaching Hospital, Lagos, Nigeria.

2.1 Sources and identification of Isolates

Ninety seven women attending the cytology clinic of Lagos University Teaching Hospital (LUTH), a nearby community clinic and students of the LUTH school of nursing were divided into three groups on the basis of their Nugent score into BV (Nugent score 7-10), Intermediate (4-6) and Negative (1-3). Ninety seven isolates of lactobacillus were cultured from high vaginal swabs from the women and stored on 10% glycerol in Brain -Heart infusion broth. Isolates were subcultured from glycerol broth onto MRS agar and incubated at both 37⁰C in 5-10% carbon dioxide and anaerobically for 24-48 hours. All isolates were identified as lactobacillus species if they grew on MRS agar, were gram positive bacilli and catalase negative.

2.2 Quantitative Analysis of Hydrogen Peroxide

The test is based on the principle that each ml of 0.1N potassium permanganate is equivalent to 1.070mg of hydrogen peroxide and therefore at the endpoint the volume of potassium permanganate used is equivalent to the H₂O₂ produced. To 5ml of broth culture of each test organism, 5ml of 0.1M dilute sulphuric acid was added and titrated with 0.1N potassium permanganate. The endpoint was reached when the sample was decolourised. The negative control was broth without lactobacilli. The volume of hydrogen peroxide produced by the organism was then compared with the Nugent score in the source. Means were compared using the Student's T test. A p value of < 0.05 was considered significant

3. RESULTS AND DISCUSSION

Fifteen of the 97 women studied had BV. Of the 97 lactobacilli isolated, 76 (78%) were facultative anaerobes, while 21 (22%) were strict anaerobes. Of the 76 facultative anaerobes 11 were obtained from women with BV while 65 were from women without BV (Table 1).

Table 1: Facultative anaerobes and anaerobic lactobacilli versus Nugent score in women with and without BV

	Negative Score (n=33)	Intermediate Score (n = 49)	BV score (n= 15)	Total
Facultative Lactobacilli	28 (85%)	37 (76%)	11 (73%)	76 (78.35)
Anaerobic Lactobacilli	5 (15%)	12 (24%)	4 (27%)	21 (26.58)

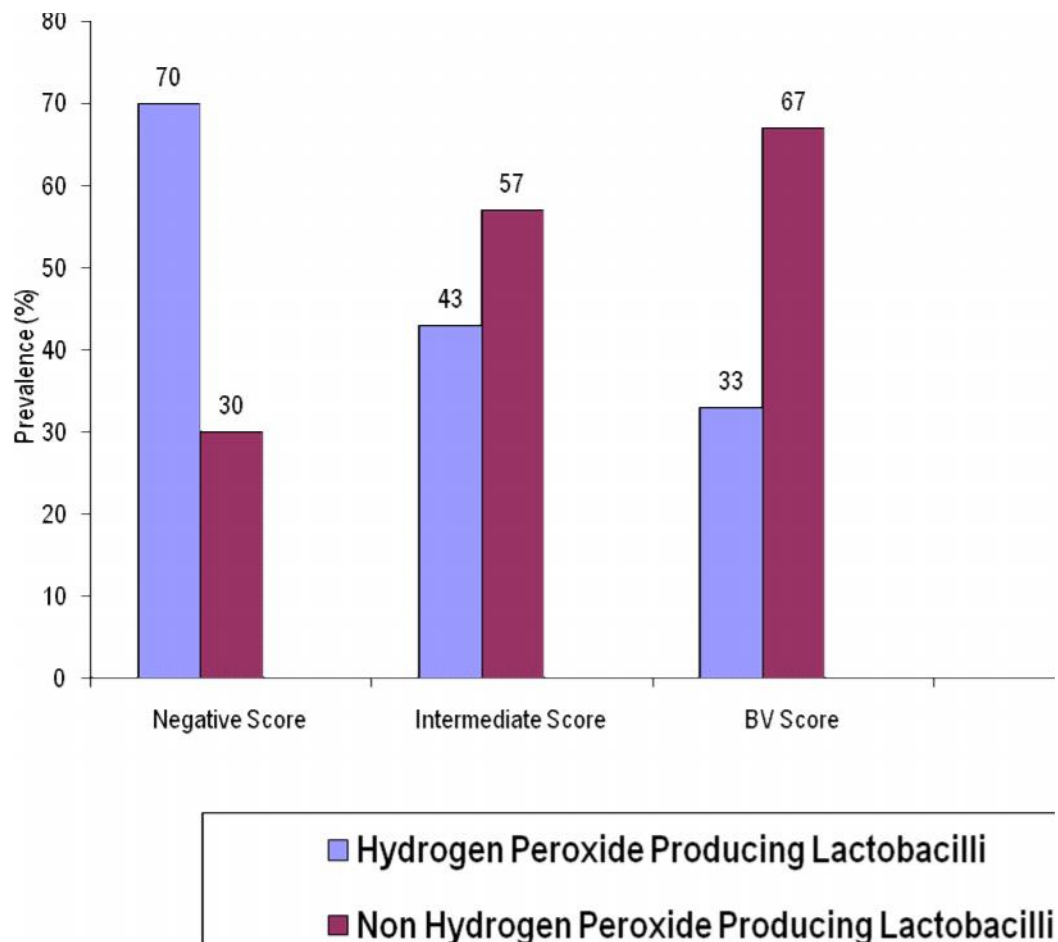
Forty nine (50.51%) of the 97 isolates produced H₂O₂ and forty four (89.79%) of these from women without BV (Table 2). The proportion of H₂O₂ producers by Nugent score were 70%, 43% and 33% in negative, intermediate and BV Nugent scores respectively (Table 2).

Table 2: Rate of production of hydrogen peroxide by lactobacilli in women with and without BV

	No BV (82)		BV (n= 15)	
	Negative (n=33)	Score Intermediate score (n = 49)		
Facultative Lactobacilli	18	13	4	35(71.42%)
Anaerobic Lactobacilli	5	8	1	14(28.57%)
Total H ₂ O ₂ producers	23 (70%)	21 (43%)	5 (33%)	

As shown on figure 1, majority of the strains obtained from women with BV did not produce hydrogen peroxide. Figure 1 also shows that prevalence of H₂O₂ producing lactobacilli increased with Nugent score, whereas, that of non-H₂O₂ producers decreased with Nugent score.

Fig 1: Histogram comparing prevalence of H₂O₂ and non- H₂O₂ producing Lactobacilli with Nugent score in women with and without BV.



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127 There was no statistically significant difference in the mean concentrations of H₂O₂ produced by the obligate anaerobes
 128 with respect to Nugent scores. Although the mean concentration of H₂O₂ produced by facultative lactobacilli in the
 129 intermediate was significantly higher than the negative score, this did not correlate with presence or absence of BV
 130 (Tables 3 and 4).

Table 3: Correlation of mean concentrations of H₂O₂ produced by Facultative Lactobacilli with Nugent scores using T-test.

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	Negative Score	Intermediate Score	BV Score
	(n = 18)	(n=13)	(n = 4)
Mean	0.1415	0.2586	0.1659
P. value	-	< .05	> .05

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135 **Table 4: Correlation of mean concentrations of H₂O₂ produced by Anaerobic Lactobacilli with Nugent scores**
 136 **using Student's T-test.**

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	Negative Score	Intermediate Score	BV Score
	(n = 5)	(n=8)	(n = 1)
Mean	0.1648	0.1511	0.0535
P. value	-	> .05	> .05

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140 DISCUSSION

141 In this study there was a much proportion of the facultative anaerobes regardless of association with BV, which is in
 142 keeping with some previous reports³. As many as 14 (66.67%) of the 21 anaerobic lactobacilli in this study produced
 143 H₂O₂. Although, this appears unusual since obligate anaerobes did not produce H₂O₂ in previous studies. Whittenburg in
 144 1964 had demonstrated that there was no correlation between H₂O₂ production and a preference for anaerobic or aerobic
 145 growth conditions^{3, 4}. Eschenbach *et. al.*, in 1989 however mentioned the possibility of the obligate anaerobes transiently
 146 producing small amounts of H₂O₂³. It is therefore noteworthy that all the anaerobic lactobacilli isolated from women with
 147 negative Nugent scores produced H₂O₂ while most of the ones isolated from women with BV scores (20%) did not. Of
 148 particular note is the fact that the women with intermediate scores provided the highest number of lactobacilli isolated
 149 many of which were obligate anaerobes, which constituted half of all obligate anaerobes isolated. These anaerobes
 150 nevertheless produced H₂O₂ but at a lower rate than in the negative scores. If indeed anaerobes only produce H₂O₂
 151 transiently, making carriers susceptible to BV, it could be that, for some reason, facultative anaerobes become replaced
 152 by obligate anaerobes which produce H₂O₂ that cannot sustain protection in the women, causing the flora to gradually
 153 progress to that of BV. It also appears there were other factors that protected the vagina from BV where H₂O₂ was not
 154 present. Only 50% of all isolates produced H₂O₂ and as many as 30% of isolates from 0-3 Nugent (negative) scores did
 155 not. This is worrying because it may imply that many women are susceptible to BV, especially since the non - H₂O₂
 156 producing Lactobacilli were isolated more frequently from women with intermediate scores (57%) and BV scores (67%),
 157 probably confirming that the absence of H₂O₂ producing Lactobacilli constitute a primary defect which increases
 158 susceptibility of the vagina to overgrowth by pathogenic^{3,9,10} present in BV.

159 Studies have shown that the presence of H₂O₂producing lactobacilli in the vagina protects prevents it from BV. This study
 160 confirms that H₂O₂ producing lactobacilli generally protects against BV while non H₂O₂ producers bacilli do not (figure 1).
 161 Nevertheless, H₂O₂ producing lactobacillus failed to prevent BV in 33% of the women (Table 2). There are probably

factors that determine in vivo production and effectiveness that need to be understood especially since this study shows that the concentration of H₂O₂ produced has no correlation with the presence or absence of BV. It is not certain that probiotic use of lactobacillus or topical use of H₂O₂ will be effective in such women.

4. CONCLUSION

The overall rate of hydrogen peroxide production was low. Nigerian women may thus have a relatively high susceptibility rate to vaginal infections. While the rates of hydrogen peroxide production correlated with Nugent scores, being highest in negative Nugent scores and lowest with BV scores, the concentration of hydrogen peroxide produced had no association with Nugent scores. There is a need to study the factors that determine the production and effectiveness of H₂O₂ produced by lactobacillus in vivo.

AUTHORS' CONTRIBUTIONS

“Author A” designed the study and wrote the first draft of the manuscript, “Author B” performed the statistical analysis, “Author B” and “Author C” managed the literature searches, “Author B” and “Author E” managed the analysis of the study, “Author D” wrote the protocol. All authors read and approved the final manuscript.

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