



(RESEARCH ARTICLE)



EVALUATION OF VIRULENCE GENES (*ICA* AND *FNB*) ASSOCIATED WITH PRETERM LABOUR IN TERTIARY HOSPITALS, BAYELSA STATE, NIGERIA

Tolulope Olukemi. Alade ^{1,*}, Daw Mercy Omeremu ² and Tاتفeng Youtchou Mirabeau ¹

¹ Department of Medical Laboratory Science, Faculty of Basic Medical Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria.

² Department of Medical Laboratory Science, Bayelsa State College of Health Technology, Nigeria.

* Corresponding Author; E.mail: tolulopealade@ndu.edu.ng

ORCID Details

Tolulope Olukemi. Alade: <https://orcid.org/0000-0002-1058-8333>

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ABSTRACT

Background: Preterm Labour is when the uterus starts contracting regularly and the cervix begins to thin and open before it reaches 37 weeks of gestation. Preterm labour is caused by a Gram-positive bacterium, Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, which is one of the main causes of newborn infections, especially sepsis and meningitis.

Aim: The study aimed at evaluating virulence genes (*ica* and *Fnb*) associated with preterm labour in Tertiary Hospitals, Yenagoa, Bayelsa State, Nigeria. A total of 168 high vaginal swabs, of which 60(35.7%) 26(15.5%) and 82(48.8) were from Family Support Program Clinics (FSP), Federal Medical Centre Yenagoa (FMCY), Diete Koki Memorial Hospital (DKMH), respectively. The samples were aseptically cultured on agar plates and incubated at 37°C for 18-24hrs, using Microbiological standard procedures for the purpose of isolating pathogenic microbes responsible for preterm labour. Virulence genes such as (*fnba* and *biofilm* gene) were detected using molecular methods. Associations were assessed using chi-square tests at $p \leq 0.05$.

Results: The various bacteria isolated were *Staphylococcus aureus* 21(18.3%), *Streptococcus sp* 2(1.7%), *Bacillus sp* 5(4.3%), *Coagulase negative staph* 1(0.9%), *Escherichia coli* 11(9.6%), and a fungus *Candida sp* 75(65.2%). However, FSP recorded a total of 28(68.2%) microbial isolates, FMCY 12(60.0%) microbial isolates and D KMH 35(64.8%) microbial isolates. Participants between 15–35 years, were in early third trimester (28–33 weeks) and were of low parity. *Candida spp.* was the predominant isolate across all institutions, age groups, gestational ages, parity levels, and behavioral practices, followed by *Staphylococcus aureus* and *Escherichia coli*. Microbial isolation clustered mainly at 28–33 weeks gestation. Low-parity women accounted for most infections. Vaginal douching and sex toy use were associated with altered microbial patterns, particularly increased *Candida* colonization and selective recovery of less common opportunistic pathogens, although most associations with age were not statistically significant.

Conclusion: Pathogenic microbes, amongst participants between 15–35 years, has overpowered the genital tract of pregnant women, hereby causing an infection. Health education and the strengthening of antimicrobial stewardship, should be encouraged to reduce infection-related preterm labour and improve maternal and neonatal outcomes.

Keywords: Virulence genes, Preterm labour, Pathogenic organisms, gestational.

1 INTRODUCTION

One of the main causes of newborn infections, especially sepsis and meningitis, is known to be Group B Streptococcus (Steven et al., 2019). Research from a number of sub-Saharan African nations, such as Zimbabwe, Malawi, Kenya, and The Gambia, has shown that GBS infections are increasingly contributing significantly to infant morbidity and death in the area. GBS-related neonatal infections are often divided into two categories: early-onset disease (EOD) and late-onset illness (LOD). Early-onset illness typically manifests soon after birth and can be caused by germs passing through the placental membranes during pregnancy or by the infant breathing in infected vaginal secretions during delivery. Low-birth-weight and premature babies are more susceptible to this kind of illness. On the other hand, progressive bloodstream infections are frequently linked to late-onset illness, which can manifest weeks or even months after delivery (Akinlolu et al., 2018). Preterm birth occurs 8–11% of pregnancies worldwide, while the precise percentage varies according to geographic location, genetic variables and socioeconomic circumstances. Nearly 75–80% of infant fatalities globally are caused by it, making it one of the biggest causes of neonatal morbidity and mortality (Noble & Deepak, 2022; WHO, 2023).

The group of microorganisms that naturally live in the vaginal environment is known as the vaginal microbiota. Together, these organisms which are mostly bacteria form the vaginal microbiome. *Lactobacillus* species, which support the maintenance of a stable and protective ecology within the vagina, often dominate this habitat in healthy women. They play a crucial role in preserving the acidic condition of the vagina, which helps stop the growth of harmful infections. In a healthy vaginal ecology, *Lactobacillus* species predominate, although other bacteria including *Prevotella* sp, *Streptococcus* sp, and *Corynebacterium* can also be found (Dos Santos et al., 2024). By generating a number of antibacterial compounds, including lactic acid, hydrogen peroxide, and bacteriocins, *Lactobacillus* species support vaginal health. Lactic acid production keeps the pH of the vagina between 3.5 and 4.5, which prevents potentially dangerous germs from growing. The vaginal microbial makeup may change to a more diversified community dominated by anaerobic bacteria as the number of *Lactobacillus* species declines. Bacterial vaginosis (BV) is often linked to this microbial imbalance, which is generally categorized as community state type IV (CST IV) (Dong et al., 2024).

2 MATERIALS AND METHODS

2.1 Study Area

The study area consist of Federal Medical Centre Yenagoa (FMCY), Diete Koki Memorial Hospital (DKMH), and Family Support Program (FSP) Clinic all in Yenagoa, Bayelsa State, Nigeria.

2.2 Study Population and Sampling Technique

All consenting pregnant women between the gestational ages 28-40 weeks are the study population. It was a cross-sectional observational study design, and the sample size was determined based on a convenient random sampling, of which, a sample size of 168 high vagina swab were used for this study.

2.3 Sample size determination

Sample size was determined using Cochran Formula, $n = \frac{Z^2 pq}{d^2}$

2.4 Inclusion/Exclusion Criteria

For this study, all willing pregnant women between the gestational ages of 28 and 40 were used. Excluded were pregnant women under 28 week's gestation and those who had taken antibiotics at the prenatal clinic?

2.5 Ethical Consent.

Ethical clearance was collected from Federal Medical Centre.

2.6 Sample Analysis

Cultural method- High vagina swab samples were aseptically cultured on Blood agar plates, Chocolate agar, as well as selective media called Nalidixic acid blood agar, following microbiological standard procedures. Plates were incubated overnight at 37°C for bacteria growth.

2.7 Molecular Analysis

2.7.1 DNA extraction

DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep kit (Inqaba, South Africa). A dense culture of purified bacteria was suspended in 200 µL of isotonic buffer within a ZR Bashing Bead Lysis tube, followed by the addition of 750 µL of lysis buffer to facilitate cell disruption. The mixture was then agitated in a bead-beating device at maximum speed for approximately five minutes to ensure complete lysis. The resulting lysate was centrifuged at 10,000 × g for one minute, and 400 µL of the supernatant was transferred to a Zymo-Spin IIF spin filter in a collection tube and centrifuged again at the same speed to collect the filtrate. To this filtrate, 1,200 µL of DNA binding buffer was added, and 800 µL of the mixture was loaded onto a Zymo-Spin IIC column, centrifuged, and the flow-through discarded. The remaining mixture was then processed through the same column under identical conditions. For purification, 200 µL of DNA Pre-Wash buffer was applied and centrifuged at 10,000 × g, followed by 500 µL of fungal/bacterial DNA wash buffer under the same conditions. Finally, the column was placed in a clean 1.5 mL tube and 100 µL of DNA elution buffer was added to release the purified DNA, which was stored at –20 °C for downstream molecular analyses.

2.7.2 DNA quantification

The concentration of the extracted genomic DNA was determined using a Nanodrop 1000 spectrophotometer. To begin, the instrument's software was launched by double-clicking the Nanodrop icon. Before measuring the DNA, the device was initialized with 2 µL of sterile distilled water and calibrated (blanked) using normal saline to ensure accurate readings. Subsequently, 2 µL of the extracted DNA sample was carefully placed on the lower pedestal, after which the upper pedestal was gently lowered to make contact with the sample. The DNA concentration was then assessed by selecting the “measure” function within the software, which provided the quantitative readout. This procedure ensures precise quantification of DNA while minimizing sample loss or contamination.

2.8 Molecular Detection of Virulence Genes

2.8.1 Biofilm Gene Amplification

Biofilm genes were amplified using Biofilm F (5'-GATTCAATTTTGGCGATTCCTGC-3') and Biofilm R (5'-TAATGAAGTCATTCAGACTCATCC-3') primers. Reaction conditions: 30 µL PCR mix, 50ng template, 0.4 µM primers, 35 cycles with 5 min initial denaturation, 1 min denaturation, 1 min annealing (47°C), 2 min extension (72°C), and 10 min final extension. Products were visualized on a 1% agarose gel at 200 V for 15 min.

2.8.2 Fibronectin-binding protein A (FNBA) Gene Amplification

FNBA genes were amplified using primers FNBA F (5'-CATAAATTGGGAGCAGCATCA-3') and FNBA R (5'-ATCAGCAGCTGATTCCTCAT-3') in 30 µL reactions under similar conditions: 50ng template, 0.4 µM primers, 35 cycles of 5 min initial denaturation, 1 min denaturation, 1 min annealing (46°C), 2 min extension, and 7 min final extension. Products were visualized on 1% agarose gel at 200 V for 15 min.

2.8.3 16SrRNA Amplification

Universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') were used to amplify the 16SrRNA gene in 40 µL reactions containing DreamTaq Master Mix, 0.5 µM primers, and DNA template. Cycling: initial denaturation (95°C, 5 min), 35 cycles of 95°C for 30 s, 52°C for 30 s, 72°C for 30 s, and final extension 72°C for 5 min. Products were separated on 1% agarose gel at 200 V for 15 min.

2.8.4 Intercellular Spacer (ITS) Amplification

Fungal ITS regions were amplified using ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers in 50 µL reactions with DreamTaq Master Mix and 0.4 µM primers. PCR cycles included initial denaturation (95°C, 5 min), 35 cycles of 95°C (30 s), 53°C (30 s), 72°C (30 s), and a final extension at 72°C for 5 min. Products were separated on 1% agarose gel at 120 V for 15 min and visualized under blue light.

2.8.5 Sequencing of PCR Products

Purified PCR products were sequenced using the BigDye Terminator v3.1 kit on an ABI 3510 sequencer (Inqaba Biotech). Each 10 µL reaction contained 0.25 µL BigDye, 2.25 µL sequencing buffer, 10 µM primer, and 2–10ng template per 100bp. Thermal cycling: 32 cycles of 96°C (10 s), 55°C (5 s), 60°C (4 min)

2.9 Statistical Analysis

Data were analyzed in SPSS v30. Associations between bacterial isolates and their geographic origin were tested using chi-square analysis. P-values < 0.05 were considered statistically significant.

3 RESULTS

Table 1a: Demographic Presentation of the Participants by Health Institutions.

Age	FSP (N=60) (%)	FMC (N=26)(%)	DIETE KOKI (N=82) (%)	Total (N=168)(%)
15-25	29(48.3)	9(34.6)	39(47.6)	77(45.8)
26-35	28(46.7)	15(57.7)	37(45.1)	80(47.6)
36-45	3(5.0)	2(7.7)	6(7.3)	11(6.55)
Total	60(35.7)	26(15.5)	82(48.8)	168(100)
Gestational Age (Weeks)				
28-30	32(53.3)	12(46.2)	27(32.9)	71(42.3)
31-33	12(20.0)	9(34.6)	25(30.5)	46(27.4)
34-36	16(26.7)	3(11.5)	21(25.6)	40(23.8)
37-38	0(0.0)	2(7.7)	9(11.0)	11(6.5)
Total	60(35.7)	26(15.5)	82(48.8)	168(100)

Table 1b: Demographic Presentation of the Participants by Health Institutions.

Parity	FSP (N=60)(%)	FMC (N=26)(%)	DIETE KOKI (N=82) (%)	Total (N=168)(%)
1	25(18.3)	11(42.3)	40(48.7)	76(45.2)
2	27(68.3)	11(42.3)	26(31.7)	64(38.1)
3	3(5.0)	2(7.7)	9(11.0)	14(8.3)
4	3(5.0)	2(7.7)	5(6.1)	10(6.0)
5	2(3.3)	0(0.0)	2(2.4)	4(2.4)
Total	60(35.7)	26(15.5)	82(48.8)	168(100)
Hygiene				
Douching	18(30.0)	10(38.5)	29(35.4)	57(33.9)
Not Douching	42(70.0)	16(61.5)	53(64.6)	111(66.1)
Total	60(35.7)	26(15.5)	82(48.8)	168(100)
Sexual Practice				
Sex Toy	16(26.7)	9(34.6)	22(26.5)	47(28.0)
No Sex Toy	44(73.3)	17(65.4)	60(73.2)	121(72.0)
Total	60(35.7)	26(15.5)	82(48.8)	168(100)

Table 2: Frequency Distribution of Microbial Isolates by Health Institution.

LOCATION ORGANISM	FSP (N=60)	FMC (N=26)	Diete Koki (N=82)	Total N=168	χ^2	p
<i>Staph aureus</i>	7(11.7%)	6(23.1%)	8(9.8%)	21(12.5%)		
<i>Strept spp</i>	0(0.0%)	0(0.00%)	2(2.4%)	2(1.2%)		
<i>Bacillus spp</i>	2(3.3%)	0(0.00%)	3(3.7%)	5(3.0%)		
Coag -ve Staph	0(0.0%)	0(0.0%)	1(1.2%)	1(0.6%)	5.88	0.825 ^u
<i>E. coli</i>	4(6.7%)	2(7.7%)	5(6.1%)	11(6.5%)		
<i>Candida spp</i>	28(0.0%)	12(20.0%)	35(58.3%)	75(44.6%)		
TOTAL	41(24.4%)	20(11.9%)	54(32.1%)	115(68.5%)		

Table 3: Frequency Distribution of *icaA* and FNBA by isolate in Health Institutions

DKMH		
Isolates	<i>icaA</i> (%)	FNBA (%)
<i>S. aureus</i> (n=8)	3(37.5)	3(37.5)
<i>E. coli</i> (n=5)	-----	1(20.0)
<i>S. aureus</i> (n=7)	FSP 2(28.6)	3(42.9)
<i>E. coli</i> (n=4)	-----	1(25.0)
<i>S. aureus</i> (n=6)	FMCY1(25.0)	4(66.7)
<i>E. coli</i> (n=2)	----- $\chi^2 = 9.84, p = 0.02^*$	1(50.0)

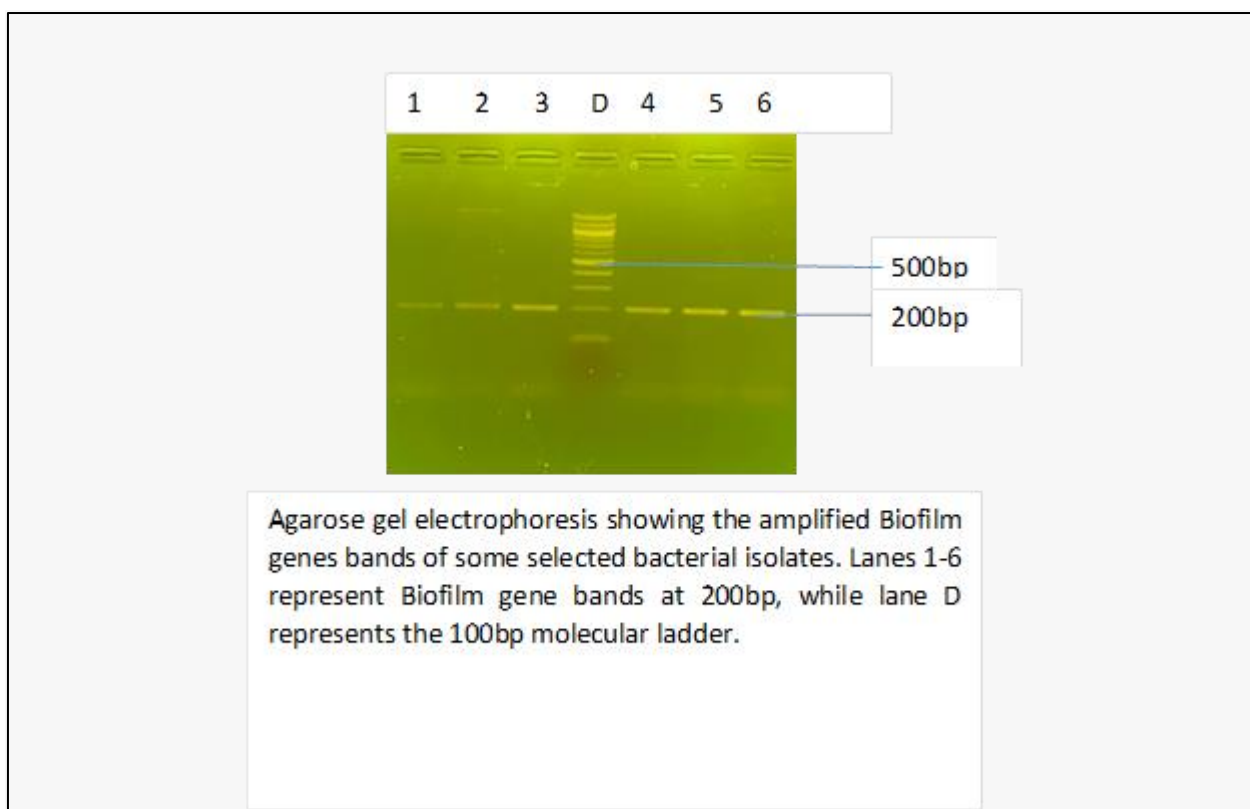


Figure 1: Biofilm (ica)

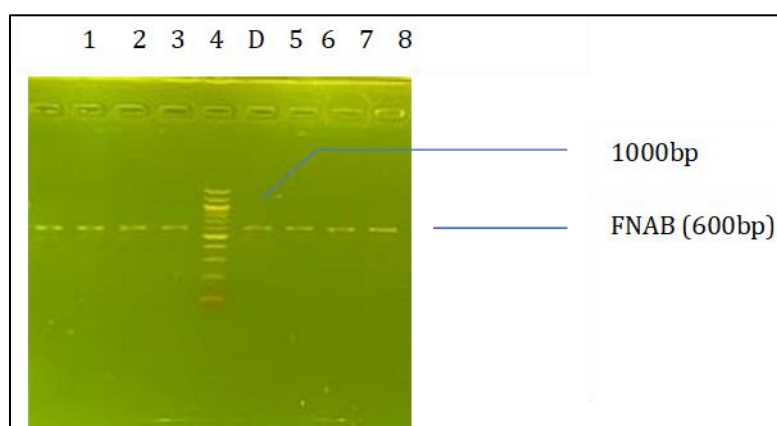


Figure 2: Agarose gel electrophoresis of some selected bacterial isolates. Lane 1-8 represent FNAB gene bands (600bp). Lane D represents the 100bp Molecular ladder.

4 DISCUSSION

High patronage, robust and service efficient antenatal care, proximity, cheaper price of medical bills and delivery items are characteristics of National Health Insurance Scheme NHIS. This aligns with Okonko *et al*, (2018), as women aged 15–30 years constituted the largest group of participants in a microbiological studies of vaginal flora, showing that this age group is both sexually active and more likely to seek care.

The highest proportion of women for gestational age were between 28–30 weeks. This shows that most participants were recruited in the early third trimester. This study aligns with Akinajo *et al*, (2019), where the majority of pregnant women who participated in their study were recruited between 28–32 weeks.

Most participants were either primiparous or secundiparous women, which aligns with Olawale *et al*, (2019), who observed that the majority of women attending antenatal clinics, were those in their first and second pregnancy, which is typical of women within the peak reproductive age group. This finding suggest that vaginal douching remains a

prevalent practice among women especially pregnant women, even though more than half do not engage in douching. This study aligns with Morris *et al.*, (2020), who documented douching prevalence rates between 25% and 40% among reproductive-aged women globally. The proportion of women reporting sex toy use is significant in the context of Nigeria, where such practices are often stigmatized. This reflects changing sexual behaviors and a growing openness toward alternative sexual practices. This is similar to findings according to Herbenick *et al.*, (2017).

There is a notable difference in microbial isolates across FSP, FMC Diete Koki. The overall prevalence of isolates across institutions was Diete Koki, recording the highest proportion followed by FSP and FMC. These institutional differences may reflect variations in patient population, diagnostic practices, hygiene conditions, and healthcare-seeking behaviors, as previously suggested by Alemu *et al.*, (2020) and Okonkwo *et al.*, (2021).

The frequency distribution of the *ica* and *fnb* by health institutions revealed that all institutions had the same distributions. Furthermore, *fnb* gene had the highest prevalence with FMCY showing a higher distribution compared to *icaA* gene. This could be as a result of geographical difference. Hence a significant association was observed between the *icaA* and *fnbA* genes and the microbial isolates, as $p > 0.02$.

5 CONCLUSION

Preterm labour remains a complex obstetric challenge and a major contributor to neonatal mortality and long-term disability. Genital tract infections and antimicrobial resistance are also major contributors to adverse pregnancy outcomes, resulting to preterm labour. This study demonstrates that pregnant women attending antenatal clinics in Yenagoa, particularly at Diete Koki Memorial Hospital, bear a substantial burden of genital tract microbial colonization.

Recommendations

- Future studies should adopt longitudinal designs to better establish causal relationships between microbial colonization, hygiene and sexual practices, antimicrobial resistance, and preterm labour outcomes.
- There is a need to strengthen antimicrobial stewardship programs within antenatal and hospital settings to promote rational antibiotic use and reduce selective pressure for resistance.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

There is no conflict of interest among the authors, the concept of the study was designed by Tاتفeng TM, the practical work was carried out by Onememu Mercy while the editing and design of the manuscript was carried out by Tolulope O. Alade.

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