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Journal of Clinical Obstetrics and Gynecology Research

ISSN 2766-2756

DIRECTIVE PUBLICATIONS

Research Article

Distribution Of Resistance Genes Erm A, B,C And Meca In Gram Positive Organisms Associated With Preterm Labour In Yenagoa, Bayelsa State, Nigeria.

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Abstract

Most major adverse pregnancy outcomes, such as preterm birth and especially preterm labour, are caused by an imbalance in the vaginal microbiome. Preterm labour which is the occurrence of frequent uterine contraction before 37 weeks of gestation, is majorly caused by a bacterium called *Streptococcus agalactiae*. The study aimed at determining resistance genes erm A, B,C and mecA associated with preterm labour. A total of 168 participants were enrolled in this study, of the 168 participants, 60(35.7%) Family Support Program Clinics (FSP), 26(15.5%) Federal Medical Centre Yenagoa (FMCY), and 82(48.8) Diete Koki Memorial Hospital (DKMH), all in Yenagoa, Bayelsa State, Nigeria. High vaginal swab samples were analyzed using standard Bacteriological technique and selected resistance genes (*emrA, emrB, emrC, mecA*,) were detected by Polymerase Chain Reaction. Socio-demographic data, gestational age, parity, hygiene practices were obtained using structured questionnaires. Chi-square tests at $p \leq 0.05$ was used for the association. 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 *Candida sp* 75(65.2%) were reported respectively. However, Microbial isolates occurrence were 28(68.2%) 12(60.0%), 35(64.8%) for FSP, FMCY and DKMH respectively. DKMH recorded the highest participation. Most participants aged 15–35 years, were in early third trimester (28–33 weeks), and of low parity (first or second pregnancy). *Candida spp*. was the most prevalent across all institutions, followed by Staphylococcus aureus and Escherichia coli. Microbial isolation clustered mainly at 28–33 weeks gestation. Low-parity women accounted for most infections. High levels of antimicrobial resistance were observed. Efflux pump genes (*emrA, emrB, and emrC*) were widely distributed across all facilities, with *emrC* being the most prevalent. The *mecA* gene was detected in over 75% of Staphylococcal isolate from FMC being mecA-positive, indicating a high burden of methicillin-resistant strains.Vaginal infections were dominated by *Candida spp*. and resistant bacterial pathogens. The widespread presence of *mecA* and efflux pump genes highlights an alarming level of antimicrobial resistance. Routine antenatal screening, strengthened antimicrobial stewardship, and targeted health education addressing harmful hygiene and sexual practices are urgently needed to reduce infection-related preterm labour and improve maternal and neonatal outcomes

Keywords: *Preterm labour, resistance genes vaginal microbiome, hygiene practices, sexual practices, parity, gestational age.*

INTRODUCTION

Preterm labour is to the occurrence of frequent uterine contraction, usually between 1 in every 5-8 minutes with cervical dilation and effacement before the completion of 37 weeks of gestation. This usually results in preterm birth (PTB). An epidemiological burden revealed that, an important insight of Preterm labour to neonatal morbidity and mortality has been highlighted globally. Approximately 1 in 10 infants is born prematurely, and nearly 900,000 diseased babies each year are linked to complications of preterm birth (WHO, 2023). This incidence is mostly common within middle-income countries, particularly in Africa and South Asia, where the rates to survival for most preterm infants remain poor due to limited neonatal care resources (WHO, 2023). Preterm labour has been an obstetric burden and a major cause to neonatal mortality and long-term disability resulting from association from infections, maternal health conditions inflammatory reactions other socio-environmental factors, Hamid, (2023). When the uterus starts contracting regularly and the cervix

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Received: 28-October-2026, Manuscript No. JOCOGR - 6332**; Editor Assigned:** 02-November-2026 **; Reviewed:** 17-November-2026, QC No. JOCOGR - 6332 **; Published:** 20-November-2026.**DOI:** 10.52338/jocogr.2026.6332.

Citation: **Tolulope O. Alade**. Distribution Of Resistance Genes Erm A, B,C And Meca In Gram Positive Organisms Associated With Preterm Labour In Yenagoa, Bayelsa State, Nigeria. Journal of Clinical Obstetrics and Gynecology Research. 2026 November; 20(1). doi: 10.52338/jocogr.2026.6332.

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begins to thin and open before it reaches 37 weeks, it is a sign of Preterm labor. These contractions often occur every five to eight minutes. One bacterium that is frequently connected to this condition is Group B Streptococcus (GBS), a gram-positive microorganism that naturally lives in the gut and vaginal tract, (Matthew et al., 2023). These bacteria, officially known as *Streptococcus agalactiae*, is distinguished from other Streptococcus species by the presence of a particular cell-wall antigen called the Lancefield group B polysaccharide (Rosa-Fraile & Spellerberg, 2017).

MATERIALS & METHODS

Study Area

This study was carried at in three health institutions namely, Federal Medical Centre Yenagoa (FMCY), Diete Koki Memorial Hospital (DKMH), and Family Support Program (FSP) Clinic all in Yenagoa, Bayelsa State, Nigeria. This research was done under strict adherence to the guidelines by the three health institutions. The target population consist of all pregnant women between the gestational ages 28-40 weeks. A cross-sectional observational study design was employed in this study. Only consenting pregnant women from gestational age 28-38 weeks were used for this study. This sample size was determined based on a convenient random sampling.

Study Population and Sampling Technique

The target population for this study consist of all pregnant women between the gestational age 28-40 weeks in DKMH, FSP and FSP. From this group, a sample size of 168 pregnant women was enrolled. This sample size was determined based on a convenient random sampling, to ensure the data is representative enough to provide meaningful insights into the research problem.

Sample size determination

Sample size was determined using Cochran Formula, n = $[Z^2pq/d^2]$

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.

Sample Analysis

Cultural method

Samples were cultured on Blood agar plates, Chocolate agar, as well as selective media called Nalidixic acid blood agar (to isolate Group B Streptococcus). Plates were incubated overnight at 37oc for bacteria growth.

Molecular Analysis

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 IIIF 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.

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.

Amplification of ermA (Erythromycin Ribosomal Methylase) genes

The *ermA* genes were amplified following a conventional PCR procedure. Each reaction, with a total volume of 30 µL, consisted of DreamTaq Master Mix (Inqaba, South Africa), 0.4 M of the forward primer (5'-AAGCGGTAAACCCCTCTGA-3') and reverse primer (5'-TTCGCAAATCCCTTCTCAAC-3'), along with 50ng of DNA template. Amplification was carried out using an ABI 9700 thermal cycler (Applied Biosystems) programmed for 35 cycles. The cycling conditions began with an initial denaturation at 94°C for 4 minutes, followed by repeated

cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 40 seconds, and extension at 72°C for 30 seconds, concluding with a final extension at 72°C for 5 minutes. To assess the amplification products, 1% agarose gel electrophoresis was performed at 200 V for 15 minutes. Visualization using a blue light imaging system confirmed the presence of the expected 260bp fragments, demonstrating successful amplification of the target ERMA sequences. This approach not only verifies the specificity of the primers but also ensures that the PCR conditions were optimized for reliable gene detection.

Amplification of ermB genes

The *ermB* genes from the isolates were amplified using the primer pair ERMB F (5'-CATTTAACGACGAAACTGGC-3') and ERMB R (5'-GGAACATCTGTGGTATGGCG-3') on an ABI 9700 thermal cycler (Applied Biosystems). The polymerase chain reaction (PCR) was performed over 35 cycles in a total reaction volume of 30 microliters. Each reaction included 50ng of extracted DNA as the template, primers at a final concentration of 0.4 µM, and the X2 DreamTaq Master Mix, which contains Taq DNA polymerase, dNTPs, and $MgCl_2$, supplied by Inqaba, South Africa. The thermal cycling conditions consisted of an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, primer annealing at 50.2°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension step was carried out at 72°C for 5 minutes to ensure complete amplification. Following PCR, the products were separated on a 1% agarose gel and visualized under a blue light imaging system, confirming the presence of the expected 300bp fragment. This approach ensures precise detection and verification of the ERMB gene amplification.

Amplification of ermC genes

The *ermC* genes from the isolates were amplified through polymerase chain reaction (PCR) using the primers ermC F (5'-AATCGTCAATTCCTGCATGT-3') and *ermC* R (5'-TAATCGTGGAATACGGGTTTG-3') on an ABI 9700 thermal cycler (Applied Biosystems). The reaction was carried out in a final volume of 30 microliters over 35 cycles. Each PCR mixture included 50ng of extracted genomic DNA as the template, primers at a final concentration of 0.4 µM, and the X2 Dream Taq Master Mix, which contains Taq DNA polymerase, dNTPs, and $MgCl_2$ (Inqaba Biotech, South Africa). The cycling program was set as follows: an initial denaturation step at 94°C for 4 minutes, followed by 35 cycles consisting of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 40 seconds, and extension at 72°C for 30 seconds, concluding with a final extension at 72°C for 5 minutes. After amplification, the PCR products were separated on a 1% agarose gel and visualized using a blue-light imaging system. The expected product size was approximately 300 base pairs, allowing confirmation of successful amplification.

MecA Gene Amplification

The detection of the methicillin resistance gene (mecA) was performed using polymerase chain reaction (PCR) with specific primers, MecA-F (5'-TGGCTATCGTGTCACAATCG-3') and MecA-R (5'-CTGGAACTTGTTGAGCAGAG-3'). Each PCR reaction was prepared in a total volume of 30 µL, consisting of 2X DreamTaq Master Mix (Inqaba, South Africa), which includes Taq DNA polymerase, deoxynucleotide triphosphates (dNTPs), and magnesium chloride ($MgCl_2$), supplemented with 0.4 µM of each primer and 50ng of template DNA. Amplification was carried out in an ABI 9700 thermal cycler (Applied Biosystems) using the following thermal profile: an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds (denaturation), 55°C for 40 seconds (annealing), and 72°C for 50 seconds (extension), and concluding with a final extension at 72°C for 5 minutes. The resulting PCR products were separated on a 1% agarose gel and electrophoresed at 200 V for 15 minutes. Visualization under blue light allowed identification of mecA-positive isolates, which were confirmed by the presence of DNA fragments approximately 300 base pairs in length. This approach provides a reliable molecular method for confirming methicillin resistance in bacterial samples, as the presence of mecA directly correlates with resistance to β-lactam antibiotics.

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.

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 2a. Susceptibility Pattern of Gram-Positive Bacterial Isolates in DKMH.

Microbial	**n**	**AS**	**BC**	**CF**	**PT**	**C**	**CP**	**CR**	**TE**	**LE**	**OF**	**GM**	**AT**
isolate	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
S. aureus.	8	3(37.5)	3(37.5)	4(50)	6(75.0)	6(75.0)	3(37.5)	5(62.5)	4(50)	6(75.0)	6(75.0)	6(75.0)	3(37.5)
Bacillus spp	3	2(66.7)	3(100)	3(100)	3(100)	2(66.7)	2(66.7)	3(100)	2(66.7)	3(100)	2(66.7)	2(66.7)	2(66.7)
Coag –ve Staph	1	1(100)	0(0.0)	0(0.0)	1(100)	0(0.0)	1(100)	0(0.0)	1(100)	1(100)	1(100)	1(100)	1(100)
Strept spp.	2	1(50.0)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50.0)
Total	14	7(50.0)	8(57.1)	9(64.3)	12(85.7)	10(71.4)	8(57.1)	10(71.4)	9(64.3)	12(85.7)	11(78.6)	11(78.6)	7(50.0)

Table 3b. Susceptibility pattern of Gram-positive bacterial isolates in Family Support Program Clinic.

Microbial isolate	**n**	**AS**	**BC**	**CF**	**PT**	**C**	**CP**	**CR**	**TE**	**LE**	**OF**	**GM**	**AT**
isolate	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
S. aureus	7	4(57.1)	2(28.6)	5(71.4)	5(71.4)	4(57.1)	3(42.9)	5(71.4)	4(57.1)	6(85.7)	6(85.7)	3(42.9)	7(100)
Bacillus spp	2	0(0.0)	0(0.0)	1(50.0)	2(100)	2(100)	1(50.0)	1(50.0)	2(100)	2(100)	2(100)	2(100)	2(100)
Total	9	4(44.4)	2(22.2)	6(66.7)	7(77.8)	6(66.7)	4(44.4)	6(66.7)	6(66.7)	8(88.9)	8(88.9)	5(55.6)	9(100)
Federal Medical Center, Yenagoa													
S. aureus	6	2(33.3)	2(33.3)	1(16.7)	4(66.7)	3(50.0)	6(100)	1(16.7)	4(66.7)	5(83.3)	4(66.7)	5(83.3)	2(33.3)

Diete Koki Memorial Hospital (DKMH)				
ISOLATES	*ermA (%)*	*ermB (%)*	*ermC (%)*	*mecA (%)*
S. aureus (n=8)	2(25.0)	2(25.0)	3(37.5)	6(75.0)
Bacillus spp. (n=3)	0(00)	0(00)	1(33.3)	-----
Strept sp (n=2)	1(50.0)	0(00)	0(00)	2(100)
Coag -ve staph (n=1)	0(00)	0(00)	1(100)	1(100)
Total (n=14)	3(21.4)	2(14.3)	5(35.7)	8(57.1)

Table 4.

Family Support Program (FSP)				
S. aureus (n=7)	1(14.3)	4(51.2)	5(71.4)	5(71.4)
Bacillus spp. (n=2)	1(50.0)	0(0.0)	0(0.0)	------
Total (n=9)	2(22.2)	4(44.4)	5(55.6)	5(55.6)
Federal Medical Center Yenagoa (FMCY)				
S. aureus (n=6)	6(66.7)	3(33.3)	6(66.7)	1(50.0)
$X^2 = 2.76$, $p = 0.05$ μ				

Frequency Distribution of emrA, B, C and mecA by isolate in Health Institutions.

DISCUSSION

There is high patronage, robust service efficient antenatal care, proximity and moderately low cost of medical bills and delivery items in the 3 institutions. (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. **Table 1**

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 suggests 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 this part 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). **Table 1b**

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). There was a high level of susceptibility to PT, LE, OF, and GM, with total susceptibility rates of 85.7%, 85.7%, 78.6%, and 78.6%, respectively, at Diete Koki Memorial Hospital. This suggests that these antibiotics remain relatively effective against Gram-positive organisms in this setting. This findings align with (Olowe et al., 2021) who reported that Fluoroquinolones and Aminoglycosides demonstrated strong activity against Gram-positive isolates. *Staphylococcus aureus* showed high susceptibility to PT, C, LE, OF, and GM (75.0% each) At the Family Support Program Clinic, susceptibility was highest for LE and OF (88.9%) and reached 100% for AT, suggesting these antibiotics may be highly effective in this facility. However, reduced susceptibility to BC (22.2%) and CP (44.4%) indicates possible resistance trends. Notably, S. aureus showed complete susceptibility to AT (100%), contrasting with findings in (Okeke *et al*., 2021). where resistance to similar agents has been documented. In Federal Medical Center, Yenagoa, S. aureus exhibited complete susceptibility to CP (100%) but showed markedly low susceptibility to CF and CR (16.7%). The distribution of *erm* genes among bacterial isolates showed that *ermA gene* had the highest prevalence at FMC compared to Diete Koki and FSP. This suggests that isolates from FMC may have a higher tendency to carry *emrA*, which is a component of the *ermAB-TolC* efflux pump, known to confer resistance to antibiotics such as Fluoroquinolones and Tetracyclines.

ermB gene have the highest distribution in FSP compared to FMC and Diete Koki which suggests local variation in selective antibiotic pressure, as *emrB* is directly involved in multidrug efflux and contributes significantly to antimicrobial resistance in Gram-negative bacteria as reported by Kobayashi *et al*., (2021).

EmrC gene showed the highest overall prevalence, most prevalent in FMC followed by FSP and Diete Koki. The high proportion highlights its potential role in widespread multidrug resistance, particularly since *emrC* has been associated with enhanced tolerance to detergents, dyes, and multiple classes of antibiotics.

Staphylococcus aureus and *Streptococcus spp* harboured erm A with and respectively, only *Staphylococcus aureus* harboured erm B, *Staphylococcus* aureus, *Bacillus spp* and *Coagulase negative Staphylococcus* harboured ermC respectively while *Staphlococcus aureus* and *Streptococcus species* harboured mec A gene. Mec A has the highest distribution of 57% in contrast with 32.14% from a study carried out at Nzukka, Nigeria (Martina et al., 2024)while Erm B (10.71%) and erm C (17.86%) but erm A was not expressed.

CONCLUSION

This study highlights that vaginal infections, dominated by resistant bacterial pathogens remain a major threat to maternal health in Yenagoa, especially among young, women in early third trimester. The study revealed a high prevalence of antimicrobial resistance determinants, notably widespread carriage of mecA among staphylococcal isolates and significant distribution of efflux pump genes (*emrA, emrB, emrC*). This indicates that multidrug-resistant organisms are entrenched across all health facilities. Strengthening routine antenatal screening, improving antimicrobial stewardship, enhancing infection-control practices, and intensifying community-based health education are urgently required to reduce infection-related preterm labour and improve maternal and neonatal outcomes.

Recommendations

1. Routine microbiological screening for vaginal infections should be integrated into antenatal care, particularly for women in the early third trimester and those of low parity, to enable early detection and timely management of infections associated with preterm labour.
2. Health education programs should be strengthened to discourage harmful hygiene practices such as vaginal douching and to promote safe, hygienic sexual practices, including proper cleaning and non-sharing of sex toys. These interventions should be culturally sensitive and incorporated into routine antenatal counseling.
3. Molecular surveillance of antimicrobial resistance genes should be expanded beyond research settings and incorporated into public health monitoring systems to guide empirical therapy and infection control policies in maternal healthcare facilities.

Contribution to Knowledge

1. This study has added several important contributions to existing knowledge on maternal genital tract infections, antimicrobial resistance, and preterm labour among pregnant women in Nigeria, particularly in Bayelsa State.
2. This study provides institution-specific epidemiological data on the distribution of vaginal microbial isolates among pregnant women attending antenatal clinics in Yenagoa. By comparing three different health institutions (Federal Medical Centre, Diete Koki Memorial Hospital, and Family Support Program), the study highlights how healthcare access, affordability, and institutional practices influence microbial prevalence and resistance patterns, an area that has been sparsely documented in the Niger Delta region.
3. This research expands knowledge on the relationship between maternal demographic factors (age, gestational age, and parity) and vaginal microbial colonization in pregnancy. The consistent predominance of infections among women aged 15–35 years and those in early third trimester reinforces the clinical importance of this reproductive age group, while also providing localized evidence that complements global and regional reproductive health literature.

Acknowledgements

We sincerely appreciate the study participants for their cooperation, the healthcare personnel and the three hospitals who help with the sample collection, my supervisors for their expertise.

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