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ANTIBIOTIC SUSCEPTIBILITY PROFILE OF ENTEROBACTERIACEAE ISOLATED FROM PREGNANT AND NON-PREGNANT WOMEN ATTENDING ABRAKA GENERAL HOSPITAL, NIGERIA

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Abstract

This study investigated the antibiotic susceptibility profile of Enterobacteriaceae isolated from high vaginal swabs (HVS) of 50 women (30 pregnant, 20 non-pregnant) attending Abraka General Hospital, Nigeria. Samples were collected aseptically and cultured on nutrient, MacConkey, and mannitol salt agar for bacterial isolation. Biochemical tests identified isolates, and antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method against ten antibiotics, with multiple antibiotic resistance indices (MARI) calculated. Of the 50 samples, 10 (20%) yielded Enterobacteriaceae, with equal positivity rates in pregnant (6/30) and non-pregnant (4/20) groups. *Klebsiella* spp. and *Proteus* spp. each comprised 40% of isolates, followed by *Escherichia coli* (20%). In pregnant women, *Proteus* spp. (50%) dominated, followed by *E. coli* (33.3%) and *Klebsiella* spp. (16.7%), while non-pregnant women showed *Klebsiella* spp. (75%) and Proteus spp. (25%) predominance, with no *E. coli*. Isolates from pregnant women exhibited 100% resistance to tetracycline, ceftriaxone, and augmentin, with one *E. coli* isolate pan-resistant (MARI=1.0). Isolates from pregnant women showed 50% susceptibility to ciprofloxacin and streptomycin, whereas all isolates from non-pregnant women were susceptible to ciprofloxacin. Non-pregnant isolates displayed 100% resistance to cephalothin, tetracycline, ceftriaxone, augmentin, and ceftazidime, but all were sensitive to ciprofloxacin. MARI ranged from 0.4–1.0, indicating significant multidrug resistance (MDR). The study highlights distinct microbial profiles by pregnancy status and underscores the urgent need for targeted antibiotic stewardship to address MDR in Nigeria's maternal healthcare.

Keywords*: Enterobacteriaceae, Vaginal Microbiota, Antibiotic Resistance, Pregnancy, Nigeria, High Vaginal Swab*

Introduction

The vaginal microbiota is a dynamic and complex ecosystem that serves as a critical line of defense for the female reproductive tract (Ghiliof and Wessels, 2022). In a healthy state, it is predominantly colonized by *Lactobacillus* species, which maintain a protective acidic environment (pH ~3.8-4.5) through the production of lactic acid, hydrogen peroxide, and bacteriocins, thereby inhibiting the proliferation of opportunistic and pathogenic microorganisms (Shen *et al*., 2022). However, this delicate balance can be disrupted by numerous factors, including hormonal fluctuations, antibiotic use, immune suppression, and physiological changes associated with pregnancy (Tithi, 2024). Such dysbiosis can facilitate the colonization and overgrowth of pathogenic organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Proteus* species, and Group B *Streptococcus* (*Streptococcus agalactiae*), leading to symptomatic or asymptomatic

infections (Chee *et al*., 2020; Baka *et al*., 2022).

Pregnancy induces significant immunological and endocrinological alterations that directly influence the vaginal milieu (Al-Nasiry *et al*., 2020). Elevated estrogen and progesterone levels promote glycogen deposition in the vaginal epithelium, creating an enriched nutrient source that can selectively favor the growth of certain microorganisms, particularly *Candida* species (Bayar *et al*., 2020). Although a *Lactobacillus*-dominated profile is associated with healthy pregnancies, the immunomodulated state of gestation can increase susceptibility to microbial shifts and related complications (Morselli, 2024). The clinical implications are profound; for instance, vaginal colonization with Group B *Streptococcus* is a well-established risk factor for neonatal sepsis, pneumonia, and meningitis, necessitating routine screening and intrapartum antibiotic prophylaxis (Nizet and Klein, 2015). More broadly, such infections are linked to adverse obstetric outcomes including preterm labor, premature rupture of membranes, and low birth weight (Yao *et al*., 2025).

In non-pregnant women, the vaginal ecosystem, while not subject to the same degree of hormonal change, remains vulnerable to disturbances from menstruation, sexual activity, contraceptive use, and hygiene practices like douching (Bakus *et al*., 2023; Don *et al*., 2023). Organisms commonly associated with urinary tract infections, such as *E. coli* and *Proteus* species, can ascend the reproductive tract to cause conditions like pelvic inflammatory disease. Furthermore, typically commensal organisms like *Staphylococcus* and *Bacillus* species can act as opportunistic pathogens when their numbers increase or when they express specific virulence factors (Dey and Chaudhuri, 2023).

Despite a wealth of global research on vaginal microbiota, there is a notable scarcity of localized microbiological data from many secondary and tertiary healthcare facilities in Nigeria, including Abraka General Hospital in Delta State. Given the influence of unique demographic, genetic, and environmental factors on microbial composition, understanding the local epidemiological profile is essential for developing effective diagnostic and therapeutic guidelines.

This study therefore aims to identify the bacteria isolated from high vaginal swabs of pregnant and non-pregnant women attending a hospital in Abraka. By comparing the microbial profiles and colonization patterns between these two groups, this research seeks to generate evidence that can inform targeted clinical management and enhance maternal health programs within the Nigerian context.

Materials and Methods

Study centre

This study was conducted at Abraka General Hospital, Delta State, Nigeria. The facility is a secondary health facility in Ethiope East, Delta State, Nigeria.

Sample collection

A total of 50 samples were collected, comprising 30 from pregnant women and 20 from non-pregnant women, using aseptic techniques. All samples were collected using sterile swab sticks and placed in properly labeled, sterile screw-cap containers. The specimens were transported immediately to the microbiology laboratory and processed within one hour of collection to ensure optimal recovery of viable organisms.

Isolation and Identification of Organisms

Each swab was first cultured on Nutrient Agar to support broad-spectrum microbial growth, then subcultured on MacConkey Agar and Mannitol Salt Agar for the isolation of bacteria of interest. The inoculated plates for bacterial cultures were incubated aerobically at 37°C for 24–48 hours.

After incubation, all plates were examined for microbial growth. Swabs that showed no bacterial growth or that grew only colonies morphologically consistent with *Lactobacillus* species were considered to have normal vaginal flora and were excluded from the pathogenic bacterial analysis.

Antibiotic Susceptibility Testing

The susceptibility of the various isolates to ten (10) commonly prescribed antibiotics at the antenatal clinics was determined using the Kirby-Bauer disc diffusion method, as described by Bauer et al., and standardized by the Clinical and Laboratory Standards Institute (CLSI).

For the standardization of the inoculum, a method as modified by Cheesbrough (2005) was used to prepare the McFarland 0.5 turbidity standard, which was used to measure the density of bacterial cells. This was done by preparing a 1% solution of anhydrous barium chloride ($BaCl_2$). A 1% solution of sulfuric acid (H_2SO_4) was prepared. Thereafter, both barium chloride and sulfuric acid solutions were completely mixed together in a sterile conical flask to form a turbid suspension of $BaSO_4$ in a specific proportion for the McFarland turbidity standard. The resulting mixture was stored in a foil-covered screw-cap test tube as a McFarland standard at room temperature (25°C) when not in use.

A sterile swab stick was soaked in the standardized bacterial suspension and streaked on Mueller-Hinton agar plates. The antibiotic discs were aseptically placed at the centre of the plates and allowed to incubate at room temperature for 1 hour before re-incubating at 37°C for 16-18 hours. The discs used include: Cephalothin (CEP), Tetracycline (TRX), Streptomycin (S), Ceftriaxone (CEF), Ofloxacin (OFX), Augmentin (AU), Pefloxacin (PEF), Ceftazidime (CTZ), Gentamicin (CN), and Ciprofloxacin (CPX). After incubation, the diameters of the zones of inhibition were measured to the nearest millimeter (mm) using a ruler and the result of the susceptibility test was interpreted using CLSI (2020) published guidelines for agar screen test.

Multiple Antibiotic Resistance Index

The multiple antibiotic resistance index (MARI) was calculated based on the ratio of the number of antibiotics to which the isolates were resistant to from a total of ten (10) commonly prescribed antibiotics in the hospital.

Results

Isolation and Identification of Organisms

Of the 50 high vaginal swabs processed, 10 (20%) yielded growth of potentially pathogenic bacteria belonging to the Enterobacteriaceae family. The remaining 40 samples either showed no growth (sterile) or grew only normal vaginal flora, resulting in a 20% culture positivity rate for the organisms of interest in this study as shown in Tables 1 and 2.

Table 1. Isolation, Identification, and Pregnancy Status of Bacterial Isolates

Plate Label	Pregnancy Status	Gram Staining	Citrate Utilization	Indole Production	Microorganism
HVS 1 10^-3^	Pregnant	- Rod	Negative	Negative	*Proteus* spp.
HVS 2 10^-3^	Non-Pregnant	- Rod	Positive	Negative	*Klebsiella* spp
HVS 2 10^-5^	Non-Pregnant	- Rod	Positive	Negative	*Klebsiella* spp
HVS 3 10^-3^	Pregnant	- Rod	Positive	Positive	*Escherichia coli*
HVS 4 10^-3^	Pregnant	- Rod	Negative	Negative	*Proteus* spp
HVS 5 10^-3^	Non-Pregnant	- Rod	Negative	Negative	*Proteus* spp
HVS 6 10^-3^	Pregnant	- Rod	Negative	Negative	*Proteus* spp
HVS 7 10^-3^	Pregnant	- Rod	Positive	Positive	*Escherichia coli*
HVS 7 10^-5^	Pregnant	- Rod	Positive	Negative	*Klebsiella* spp
HVS 8 10^-3^	Non-Pregnant	- Rod	Positive	Negative	*Klebsiella* spp

Table 2. Prevalence of Bacterial Isolates by Pregnancy Status

Bacterial Isolate	Pregnant Women (n=30)	Non-Pregnant Women (n=20)	Total (n=10)
Klebsiella spp.	1 (16.7%)	3 (75.0%)	4 (40%)
Proteus spp.	3 (50.0%)	1 (25.0%)	4 (40%)
Escherichia coli	2 (33.3%)	0 (0.0%)	2 (20%)

Occurrence of Microorganisms

The prevalence table shows the prevalence of bacterial isolations from the ten high vaginal swab (HVS) samples. *Klebsiella* spp. and *Proteus* spp. were the most common isolates, each accounting for 40%, while *Escherichia coli* was detected in 20% of cases.

Antimicrobial Resistance Profile

The antimicrobial resistance profiles and Multiple Antibiotic Resistance (MAR) indices for the 10 isolates, by pregnancy status, are presented in Table 3. In pregnant women, isolates exhibited complete (100%) resistance to Tetracycline, Ceftriaxone, and Augmentin. One *E. coli* isolate was pan-resistant (MAR=1.0). Ciprofloxacin and Streptomycin were the most effective agents, with 50% of isolates being sensitive.

In non-pregnant women, the isolates showed complete (100%) resistance to Cephalothin, Tetracycline, Ceftriaxone, Augmentin, and Ceftazidime. In contrast, all isolates from non-pregnant women remained sensitive to Ciprofloxacin. *Klebsiella* spp. was the dominant organism in this group and displayed a consistently high MAR index of 0.8.

Table 3. Antimicrobial Sensitivity Test

ISOLATES	Preg. Status	CEP	TRX	S	CEF	OFX	AU	PEF	CTZ	CN	CPX	MARI
Proteus	Pregnant.	R	S	S	R	R	R	R	R	R	R	0.8
E. coli	Pregnant	R	R	R	R	R	R	R	R	R	R	1.0
Proteus	Pregnant	R	R	R	R	R	R	R	R	S	R	0.9
Proteus	Pregnant	R	R	S	R	S	R	S	R	S	S	0.5
Klebsiella	Pregnant	R	R	R	R	R	R	S	R	R	S	0.8
E. coli	Pregnant	S	R	R	R	S	R	S	S	S	S	0.4
Klebsiella	Not Pregnant	R	R	S	R	R	R	R	R	R	S	0.8
Klebsiella	Not Pregnant	R	R	S	R	R	R	R	R	R	S	0.8
Proteus	Not Pregnant	R	R	S	R	S	R	S	R	S	S	0.5
Klebsiella	Not Pregnant	R	R	R	R	R	R	S	R	R	S	0.8

CEP = Cephalothin, TRX = Tetracycline, S = Streptomycin, CEF = Ceftriaxone, OFX = Ofloxacin, AU = Augmentin, PEF = Pefloxacin, CTZ = Ceftazidime, CN = Gentamicin, CPX = Ciprofloxacin

Discussion

The current study investigated the antibiotic susceptibility profile of Enterobacteriaceae isolated from high vaginal swabs (HVS) collected from pregnant and non-pregnant women attending Abraka General Hospital, Nigeria. The results revealed a 20% isolation rate for Enterobacteriaceae across the 50 samples, with *Klebsiella* spp. and *Proteus spp*. each accounting for 40% of the isolates, while *Escherichia coli* comprised 20%. All isolates were Gram-negative rods belonging to the Enterobacteriaceae family, identified through biochemical tests including citrate utilization and indole production. This predominance aligns with their role as opportunistic pathogens in reproductive tract infections, potentially contributing to complications such as preterm labor in pregnant women or pelvic inflammatory disease in non-pregnant individuals.

The low culture positivity rate (20%) observed in this study indicates limited Enterobacteriaceae colonization or infection among the sampled population, with identical rates in both pregnant (6/30, 20%) and non-pregnant (4/20, 20%) groups. In pregnant women, *Proteus* spp. was the most prevalent isolate (3/6, 50%), followed by *E. coli* (2/6, 33.3%) and Klebsiella spp. (1/6, 16.7%), suggesting a higher propensity for *Proteus* and *E. coli* colonization during pregnancy, possibly influenced by hormonal changes that alter the vaginal milieu and promote glycogen deposition favorable to these organisms. In contrast, among non-pregnant women, *Klebsiella* spp. dominated (3/4, 75%), with *Proteus* spp. at 25% (1/4) and no *E. coli* detected, indicating a shift toward *Klebsiella* predominance in the absence of pregnancy-related physiological alterations. These group-specific trends highlight potential differences in microbial ecology, with pregnant women showing greater diversity (three species) compared to non-pregnant women (two species), though overall positivity remained consistent.

In comparison, a similar study by Famojuro *et al*. (2024) in Nigeria reported higher bacterial detection rates of 80.4% in HVS from pregnant women and 81.5% from non-pregnant women, with *E. coli* as the most common isolate at 27.6% overall, followed by other Gram-negative bacteria like *Klebsiella pneumoniae*, but no significant difference in positivity between groups, akin to the current findings despite the lower rate here. Another Nigerian investigation from Port Harcourt by Mike-Ogburia *et al*. (2025) found *E. coli* at 36.67%, *Klebsiella* spp. at 29.9%, and *Proteus* spp. at 13.33% among HVS isolates, without segregating by pregnancy status, but reflecting a dominance of Enterobacteriaceae similar to the overall pattern here, though with *E. coli* more prominent than in the current non-pregnant group. In Owo, Nigeria, Okiki Pius *et al*. (2015) noted a 100% positivity rate across 65 HVS samples. More recently, Ntekpere, (2025) reported a 16.7% prevalence of bacterial growth in HVS from 360 pregnant women in Akwa Ibom State, Nigeria, with *Enterobacter* spp. at 11.7% and *Klebsiella* spp. at 8.3%, aligning closely with the lower positivity and Enterobacteriaceae involvement in the pregnant group here, but lacking non-pregnant comparisons. These Nigerian studies collectively suggest that Enterobacteriaceae are common in HVS, with positivity rates ranging from 16.7-100% in other settings, potentially influenced by factors such as hygiene practices, socioeconomic status, and local antibiotic misuse, contrasting with the consistent 20% rate observed across both groups in this study.

Globally, the prevalence of Enterobacteriaceae in HVS appears lower and more variable. For instance, in a Pakistani study, only 10.2% of HVS yielded *E. coli* and 1.5% *Klebsiella* spp., with normal flora (30%) and *Candida* spp. (21.3%) predominating (Tariq *et al*., 2006), indicating lower Enterobacteriaceae involvement compared to the 20% in both pregnant and non-pregnant groups here. Similarly, an Iranian analysis reported *E. coli* in 14.13% of 460 HVS from fertile and infertile women (13.63% in fertile and 14.58% in infertile) (Safarpoor *et al*., 2020), suggesting lower overall prevalence in infertility-related contexts, though not directly comparable to pregnancy status. Worldwide meta-analyses estimate bacterial vaginosis (often involving mixed anaerobes rather than solely Enterobacteriaceae) at 20-40%, which aligns with the 20% positivity here in both groups; however, Sethi *et al*. (2025) reported a global BV prevalence in pregnant women ranging from 4.9-49%, with a pooled 24.8% in African studies, higher than the 20% Enterobacteriaceae-specific rate in pregnant women here, underscoring regional variations where African pregnant women show elevated risks due to socioeconomic and environmental factors.

The antimicrobial susceptibility testing revealed high levels of resistance among the isolates to several antibiotics, with multiple antibiotic resistance indices (MARI) ranging from 0.4 to 1.0 overall. In pregnant women, isolates exhibited complete (100%) resistance to tetracycline (TRX), ceftriaxone (CEF), and augmentin (AU), with one *E. coli* isolate being pan-resistant (MARI=1.0) and another *E. coli* showing lower resistance (MARI=0.4). *Proteus* isolates in this group varied widely (MARI 0.5-0.9), while the single *Klebsiella* had MARI 0.8. Ciprofloxacin (CPX) and streptomycin (S) were the most effective, with 50% of isolates sensitive, indicating preserved options for fluoroquinolones and aminoglycosides in pregnancy-associated infections. In non-pregnant women, isolates showed complete (100%) resistance to cephalothin (CEP), tetracycline (TRX), ceftriaxone (CEF), augmentin (AU), and ceftazidime (CTZ), with all *Klebsiella* isolates at MARI 0.8 and the single *Proteus* at 0.5. Notably, all non-pregnant isolates remained sensitive to ciprofloxacin (CPX), suggesting better efficacy of this agent compared to the pregnant group (where only 50% were sensitive). Overall, non-pregnant isolates displayed higher uniform resistance to beta-lactams (e.g., 100% to CTZ vs. 83.3% in pregnant), but lower MARI variability, potentially reflecting differences in prior antibiotic exposure or host factors. This pattern underscores emerging multidrug resistance (MDR), likely driven by selective pressure from over-the-counter antibiotic use.

In Nigeria, similar high resistance has been documented, often without full segregation by pregnancy status. A study on HVS isolates by Adegoke *et al.* (2011) reported *E. coli* resistance to ampicillin (78%), cotrimoxazole (65%), gentamicin (52%), and ciprofloxacin (56%), with 69% of isolates producing extended-spectrum beta-lactamases (ESBLs), mirroring the beta-lactam resistance seen here, particularly in pregnant *E. coli* (100% to AU, CEF). In Mike-Ogburia *et al*. (2025) from Port Harcourt, overall HVS isolates (including *E. coli*, *Klebsiella*, and *Proteus*) showed 60% resistance to ampicillin, 75% to augmentin, and 56% to nalidixic acid, with lower resistance to gentamicin (20% overall), consistent with the variable sensitivity to gentamicin (CN) in both groups here (50% sensitive in pregnant, 25% in non-pregnant). Famojuro *et al*. (2024) found Gram-negative HVS isolates

with 85.4% resistance to carbenicillin in *E. coli* and 31.6% to imipenem in *Klebsiella pneumoniae*, highlighting ESBL prevalence (25.6-30.3%) and MDR trends similar to the high MARI values in *Klebsiella* across both groups (0.8). Additionally, Ntekpere *et al*. (2025) reported 100% sensitivity to imipenem but variable resistance (35.7-57.1%) to beta-lactams like cefotaxime and nitrofurantoin in gram-negative isolates from pregnant women, differing from the complete beta-lactam resistance here but aligning with the MDR concern. These findings indicate that beta-lactam and tetracycline resistance is pervasive in Nigerian vaginal isolates, exceeding 50-80% for many agents, possibly exacerbated by self-medication.

Worldwide comparisons reveal analogous but sometimes less severe patterns. In Eritrea, Hussein *et al*. (2024) showed that Gram-negative vaginal isolates (including *E. coli*, *Klebsiella*, and *Proteus*) displayed >45% resistance to ampicillin, trimethoprim-sulfamethoxazole, and tetracycline, with *E. coli* specifically >50% resistant to these, akin to the 100% TRX resistance in both groups here. A six-year analysis in Palestine by El Aila and El Aish (2025) showed *E. coli* from various specimens (including vaginal) with 90.3% resistance to ampicillin and 78.7% to co-amoxiclav, but lower rates to colistin (6.3%) and amikacin (10.6%), suggesting preserved efficacy for some aminoglycosides unlike the variable CN resistance here (e.g., 50% in pregnant vs. 25% in non-pregnant). In Iran, uropathogenic *E. coli* from HVS exhibited 88.63% resistance to tetracycline, 79.54% to ampicillin, and 77.27% to gentamicin, with high prevalence of resistance genes like tetA and aac(3)-IV (Safarpoor *et al*., 2020), indicating genetic mechanisms driving MDR comparable to those implied in the Nigerian context, particularly for *E. coli* in pregnant women.

The high resistance observed, particularly to beta-lactams and tetracycline, links directly to Nigeria's public health crisis of antibiotic misuse, where unregulated over-the-counter sales (72.4% of pharmacies dispense without prescriptions) and self-medication (nearly 70% of respondents) drive selective pressure and MDR emergence (Esther *et al*., 2025). This is compounded by inadequate infrastructure, leading to empirical prescribing and resistance gene dissemination, as evidenced by pooled MDR prevalence of 56.4% and high ESBL genes like blaCTX-M (23%) across human, animal, and environmental sectors (Ugbo *et al*., 2025). In 2019, AMR caused 263,400 deaths in Nigeria, exceeding malaria, underscoring the urgent need for stewardship (Taiwo, 2025).

Conclusion

The results highlight a concerning burden of Enterobacteriaceae in HVS from Nigerian women, with pregnant women showing higher *Proteus* and *E. coli* prevalence alongside greater resistance diversity (including pan-resistance), while non-pregnant women exhibited *Klebsiella* dominance with more uniform beta-lactam resistance but better CPX sensitivity. Future research should incorporate molecular typing for resistance genes, explore fungal co-infections, and advocate for targeted antibiotic stewardship to curb this public health challenge.

Conflict of Interests

The author has not declared any conflict of interests.

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