West African Journal of Life Sciences. Available online at ***http://www.wajls.com*** *ISSN (Print):2992-5150. eISSN:3122-0916*
Volume 2; Issue 2: pages 9-22

Full Length Research

Prevalence of Multi Drug Resistant Bacteria in Urine of Pregnant Women in Auchi, South-Southern Nigeria

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ABSTRACT

Multidrug resistance of urinary tract pathogens constitutes an important public health problem, especially for pregnant women, who, due to their particular physiologic state, are more prone to developing urinary tract infections. The aim of the research was to investigate antibiotics and plasmid profile in multi-drug-resistant bacteria from urine sample of pregnant women (10-40 years) in Auchi, South-southern Nigeria. A cross-sectional study was conducted involving 29 pregnant women attending antenatal clinic at Hope Hospital in Auchi, South-Southern Nigeria. Urine samples were collected and cultured using standard microbiological techniques. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method. For different age group, bacteria counts were 31.57×10^3cfu/mL in participants whose ages were from 21 to 30 years and 8.00×10^3 cfu/mL for those between 10 and 20 years. Bacterial isolates discovered included *Escherichia coli, Klebsiella* spp., *Enterococcus* spp*., Bacillus* spp. and *Staphylococcus aureus*. In all samples, significant amount of bacterial growth was observed. The most frequent isolate was *Escherichia coli* (55%) while the least frequent was *Klebsiella spp.* (2%). Bacteria were found to exhibit varying degrees of resistance to commonly used antibiotics with the most resistant bacteria being *E. coli* (1.0 MRI) while the least resistance was *Bacillus spp.* (0.8 MRI). Plasmid profile revealed the presence of plasmid in *Escherichia coli, Klebsiella* spp*., Bacillus* spp. and *Staphylococcus aureus*. Antimicrobial sensitivity after plasmid curing revealed increased sensitivity to antibiotics as a result of antibiotics removal. Resistant gene *aph(2")-If* gene was detected in *Escherichia coli, Enterococcus* spp*., Bacillus* spp. and *Staphylococcus aureus*. Hemolysin gene was detected in *Escherichia coli, Enterococcus* spp. and *Staphylococcus aureus*. This study reveals a high prevalence of multidrug-resistant bacteria in the urine of pregnant women during the second and third trimesters. There is urgent need for mandatory routine microbial screenings during antenatal care.

Received Nov., 12, 2025

Received in Revised form, Dec 3, 2025

Accepted Dec. 5, 2025

Available Online Dec. 30, 2025

W. Afr. J. Life Sci. 2 (2):9-22

Keywords: Prevalence, urine, pregnancy women, Urinary tract infection, Infectious diseases and antibiotic resistance

INTRODUCTION

Microbial resistance has remained a principal public issue over the years (Salam et al.,2023*;* Adetunji *et al*.,2023; Adetunji et al.,2023) accounting for death of over 1.2 million people in 2019 (Antimicrobial Resistance Collaboration, 2022). It occurs when viruses, fungi or bacteria adapt to antimicrobial medicines or treatment, making them ineffective (Mohamed et al.,2024). The adaptation occurs through genetic changes, spontaneous mutation or deoxyribonucleic acid (DNA) transfer (Habboush and Guzman,2023). Specifically, antibiotic resistance is the type of microbial resistance that reduces the effectiveness of antibiotics against bacteria (Chinemerem et al*.,*2022). The misuse and overuse of antibiotics in humans, animals and even plants increase the prevalence of antibiotic-resistant bacteria (Salam et al.,2023).

Multidrug-resistant (MDR) bacteria are significant concern as far as the management of urinary tract infections is concerned (Salam et al*.,*2023). They limit treatment options and exacerbate the risk of complications for both mother and fetus (Vlad et al., 2025). MDR pathogens such as Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa are frequently isolated from urine samples of infected pregnant women have been demonstrated to resist commonly prescribed antibiotics including amoxicillin and ciprofloxacin (Asmad et al., 2021). Mechanisms involved in acquisition of antibiotic resistance include enzymatic destruction of drugs, alteration of target sites, reduced drug permeability, and increased efflux pumps (Vivekanandan et al*.,*2025). Horizontal gene transfer plays a critical role in spreading resistance determinants among bacterial populations (Huddlesto et al.,2025).

Conventionally, the human urinary system contains a handful of beneficial microorganisms which contribute to health and wellness as well as disease prevention (Chorbinska et al.,2023). Interestingly, these urinary tract borne microorganisms undergo significant changes during stages of pregnancy. These changes orchestrated by progesterone include increase in abundance, stability and decrease in diversity (Hu et al*.,*2023). For instance, increased concentrations of progesterone are known to cause relaxation of the smooth muscles of the urinary tract and this result in dilation of ureter and urinary stasis. This thus creates more conductive environment for bacterial to grow. This also increases the risk of urinary tract infections.

In rural and semi-urban areas of Nigeria, there is increased reliance on traditional birth owing to cultural belief, poor and uneven access to medical care due to high cost and multi-dimensional poverty (Esan et al.,2023). Given the semi-urbanity of Auchi, there was dearth of closely similar study in selected site. Besides, there was insufficient information about the prevalence of multidrug resistant bacteria in pregnant women in Auchi. The study was designed to investigate the prevalence of multi-resistant bacteria in pregnant women in Auchi, South-Southern Nigeria.

METHODOLOGY

Research Design

The research adopted a cross-sectional study that involved pregnant women attending antenatal clinic at Hope Hospital, Auchi and South-Southern Nigeria.

Research Setting

Hope Hospital is located in Auchi, Etsako West Local Government Area of Edo State in the South-Southern geopolitical region of Nigeria. The hospital provides medical services for all medical specialties including antenatal care.

Sampling techniques

The hospital was selected through convenience sampling. The pregnant women 10-40 years who were in their second and third trimesters were selected through convenience sampling, a non-probability sampling.

Inclusion & Exclusion Criteria

The inclusion criteria include being pregnant women in second and third trimesters, absence of complication, normal vital signs; blood pressure within normal acceptable rage, pulse rate within normal acceptable rage. Pregnant women between 10 to 40 years of age.

Pregnant women with underlying ailments, pregnant women in first trimester and those at high risks of medical complications were excluded.

Analytic Procedure

Sample Collection

Urine samples collected into sterile sample bottles were received from the pregnant women between 8.00 am and 10.00 am. A total of 29 urine samples from the pregnant women were immediately transported to the laboratory for microbiological analysis.

Culture

The working area was disinfected thoroughly before and after using ethanol. Cotton wool was soaked in ethanol and was used to disinfect the work bench. Materials including glass wares and nutrient agars were sterilized before use. An autoclave was used to achieve sterilization off equipment at 121ºC for 15 minutes. Enumeration of bacterial colonies was carried out to quantify the bacterial load in urine samples collected from pregnant women. This was done by introducing 19g of CLED (Cystine lactose electrolyte-deficient) agar into 500mL of distilled water. After preparing the agar solution, the flask was covered using cotton wool wrapped with foil paper and sealed using masking tape. The flask was labelled and autoclaved using the autoclave at 121°C for 15 minutes. In another conical flask, 14g of nutrient agar was prepared and autoclaved. Each urine sample was serially diluted up to 10^{-3} cfu/mL using sterilized distilled water. A 0.1 mL aliquot from appropriate dilutions was inoculated onto the already labelled petri dishes. The already autoclaved agar media was then poured into the petri dishes while observing aseptic techniques. The standard pour plate technique was carried out. After solidification of agars, the plates were incubated at 37°C for 24 hours. After incubation, colonies exhibiting different characteristic morphology were counted manually using a colony counter.

The number of colony forming units per milliliter (CFU/mL) of urine was calculated using the formula:

$$\text{CFU/mL} = \frac{Number\ of\ colonies \times Dilution\ factor}{Volume\ Plated\ (mL)}$$

A bacterial count of $\geq 10^3$ CFU/mL was considered significant for urinary tract infection, in accordance with established clinical microbiology standards.

Sensitivity & Multi-Drug-Resistant Bacteria Screening

Twenty-eight grams of the agar was to be suspended in 1000mL off distilled water. The agar was measured using specific

measurements and poured into the beaker. The beaker was then covered or wrapped in foil paper and stabilized using masking tape. It was heated to dissolve the mixture completely. Then it was introduced into the autoclave for sterilization at 121ºC for 15 minutes. After sterilization, the agar was allowed to cool a little bit and then poured into the petri dishes. The colony was picked and streaked on the agar surface using a sterile loop. The plates were incubated at 37ºC for 24 hours. The plates were observed for growth. Cultural characteristics including the shape, color, size, elevation, margin and transparency were observed. Gram staining was carried out to classify the bacterial isolates. Biochemical Tests were carried to identify and characterize microorganisms. Catalase test, citrate test, indole, oxidase, urease test and triple sugar ion tests were carried out on bacteria isolates. Antibiotics including Rocephin, Ciprofloxacin, Azithromycin, Levofloxacin, Erythromycin, Pefloxacin, Gentamycin, Ampiclox, Zinacef, Septrin, Streptomycin, Amoxicillin, Augmentin, Ofloxacin, Cefotaxime and Sparfloxacin were used for sensitivity test.

The multidrug-resistance index (MRI) was calculated using the formula

MRI = $\frac{nR}{nA}$ ---------

Where, nR is number of resistance and nA is number of antibiotics (Zhou, 2018).

Molecular Analysis

Molecular investigations of ESBL (extended-spectrum beta-lactamases)-coding gene were done by simple polymerase chain reaction (PCR) on post-curing colonies and streaked on the agar surface using a swab stick. The plates were incubated at 37ºC for 24 hours. The plate's the extracted DNA using ESBL-coding regions specific primers. Reaction cocktail used for all PCR per primer set included (Reagent Volume µl) - 5X PCR SYBR (N',N'-dimethyl-N-[4-[(E)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-N-propylpropane-1,3-diamine) green buffer (2.5), MgCl2 (0.75), 10pM DNTP (deoxyribonucleoside triphosphate) (0.25), 10pM of each forward and backwards primer (0.25), 8000U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 µl template was added. Buffer control was also added to eliminate any probability of false amplification Table below shows the primer sequence and PCR profile in amplifying each fragment. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair. Detection of possession of resistant gene in DNA was carried out.

For *Aph (2") -If* Gene Amplification and Sequencing, the isolates were cultured on blood agar plates and incubated overnight at 35°C in a 5% CO_2 atmosphere. DNA was extracted using the AxyGen amp DNA Mini Extraction Kit (Axygen, United States) according to the manufacturer's instructions. The final pure DNA was stored at −20°C until use. The nucleotide sequence of 1kb region encoding the 'aph(2")-If gene' was amplified using polymerase chain reaction.

ETHICAL CONSIDERATION

As required, the ethical certification was obtained from the Institution Research Board, Edo State University Uzuairue, Iyamho.

STATISTICAL ANAYSIS

Data analysis was carried out using statistical software SPSS. It was used to analyze numerical data and survey data. Frequencies

were done and pairwise comparisons were carried out using Analysis of Variance (ANOVA).

RESULTS

Bacteria count of isolates from urine of pregnant women in different age groups

Table 1a showed that there was a significant difference in bacteria count between age 10 to 20 years and age 21 to 30 years. There was no significant difference in bacteria count between age 21 to 30 years and age 31 to 40 years.

Bacteria count of isolates from urine of pregnant women in different gestation age

Table 1b show there was no significant difference in bacteria count between second and third trimesters.

Bacteria count among different educational level

Table 1c showed that there was no significant between pregnant women with secondary and tertiary education levels.

Bacteria count among participant with history of urinary tract infection

Table 1d showed that there was significant difference between those who had no history of urinary tract infection (UTI) and those with 2 to 3 years history of UTI.

Table 1a: Heterotrophic Bacteria Count x10^3 (Cfu/Ml) from Urine Samples of Participants.

Age (years)	Frequency (%)	No. of Participants	Media	
			NA (cfu/mL)	CLED (cfu/mL)
10-20	1 (3.4)	1	8.00 ±0.00[b]	181.00±0.00[b]
21-30	21 (72.4)	21	31.57 ±6.49[a]	34.23±7.21[a]
31-40	7 (24.1)	7	24.14±10.85[a]	38.57±12.39[a]
p-value			0.657	0.001

Key: NA = nutrient agar, CLED = cysteine lactose electrolyte-deficient agar. Groups with same alphabets are not significantly different; Different alphabet indicated significant difference (P<0.05).

Table 1b Bacteria Count among Different Gestational Periods

Gestation age	Frequency (%)	No. of Participants	Media	
			NA (cfu/mL)	CLED (cfu/mL)
2	9 (31)	9	35.88±12.16[a]	45.33±14.38[a]
3	20 (69)	20	25.85±5.65[a]	38.10±9.40[a]
p-value			0.397	0.674

Key: NA = nutrient agar, CLED = cysteine lactose electrolyte-deficient agar. Groups with same alphabets are not significantly different; Different alphabet indicated significant difference (P<0.05).

Table 1c: Bacteria Count among Different Educational Level of Participants

EL (educational level)	Frequency (%)	No. of participants	Media	
			NA (cfu/mL)	CLED (cfu/mL)
Secondary	9 (31)	9	34.33±7.51[a]	51.22±21.59[a]
Tertiary	20 (69)	20	26.55±7.06[a]	35.45±6.01[a]
p-value			0.513	0.356

Key: NA = nutrient agar, CLED = cysteine lactose electrolyte-deficient agar. Groups with same alphabets are not significantly different; Different alphabet indicated significant difference (P<0.05)

Table 1d: Bacteria Count among Participants with History of Urinary Tract Infection

FUC (underwear change)	Frequency (%)	No. of participant	Media	
			NA (cfu/mL)	CLED (cfu/mL)
NONE	12 (41.4)	12	19.66±5.33[a]	24.66±7.15[a]
DAILY	16 (55.2)	16	35.18±8.62[a]	49.50±12.26[a]
2-3	1 (3.4)	1	41.00±0.00[b]	82.00±0.00[b]
p-value			0.353	0.181

Key: FUC = frequency of underwear change, NA = nutrient agar, CLED = cysteine lactose electrolyte-deficient agar. Groups with same alphabets are not significantly different; Different alphabet indicated significant difference (p<0.05)

Cultural characteristics of bacterial isolates from urine of pregnant women

Table 2a showed the cultural characteristics such as shape, colour, size, elevation, margin and transparency of bacterial isolates from urine of pregnant women.

Morphological characteristics of bacterial isolates from urine of pregnant women

Table 2b showed the morphological characteristics of bacterial isolates from urine of pregnant women such as cell type and cell arrangement.

Biochemical Characteristics of Isolates from urine of pregnant women

Table 2c showed the biochemical characteristics of bacterial isolates from urine of pregnant women.

Oxidative fermentation test of Isolates from urine of pregnant women

Table 2d showed the fermentation test of isolates from urine of pregnant women.

Table 2a: Cultural Characteristics of Bacterial Isolates from Urine of Pregnant Women

Parameters	H1	H2	H3	H4	H5	H6	H7
Shape	Circular	Circular	Circular	Circular	Circular	Irregular	Circular
Colour	Yellow	Yellow	Yellow	Cream	Cream	Cream	Yellow
Size	Small	Small	Medium	Small	Medium	Large	Medium
Elevation	Raised	Raised	Raised	Raised	Raised	Flat	Raised
Margin	Entire	Entire	Entire	Entire	Entire	Undulate	Entire
Transparency	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque

H1: Escherichia coli, H2: Escherichia coli, H3: Klebsiella spp., H4: Enterococcus spp., H5: Escherichia coli, H6: Bacillus spp., H7: Staphylococcus aureus

Table 2b: Morphological Characteristics of Bacterial Isolates from Urine of Participants

Parameters	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**
Gram stain	-	-	-	+	-	+	+
Cell type	Rod	Rod	Rod	Cocci	Rod	Rod	Cocci
Cell arrangement	Single	Single	Single	Single	Single	Single	Clusters

Where; + is positive, - is negative.H1: Escherichia coli, H2: Escherichia coli, H3: Klebsiella spp., H4: Enterococcus spp., H5: Escherichia coli, H6: Bacillus spp., H7: Staphylococcus aureus

Table 2c: Biochemical Characteristics of Isolates from Urine of Pregnant Women

Parameters	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**
Indole	+	+	-	-	+	-	-
Citrate	-	-	+	-	-	+	+
Catalase	+	+	+	-	+	+	+
Oxidase	-	-	-	-	-	+	-
Urease	-	-	+	-	-	-	+
H_2S	-	-	-	-	-	-	-

Where; + is positive, - is negative.H1 Escherichia coli, H2: Escherichia coli, H3: Klebsiella spp., H4: Enterococcus spp., H5: Escherichia coli, H6: Bacillus spp., H7: Staphylococcus aureus.

Table 2d: Oxidative Fermentation Test of Isolates from Urine of Pregnant Women

Parameters	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**
Glucose	A/G	A/G	A/G	A	A/G	A/G	A
Sucrose	A/G	A/G	A/G	A	A/G	A/G	-
Lactose	A/G	A/G	A/G	A	A/G	-	-
Probable isolates	*Escherichia coli*	*Escherichia coli*	*Klebsiella spp.*	*Enterococcus spp.*	*Escherichia coli*	*Bacillus spp.*	*Staphylococcus aureus*

Where; + is positive, - is negative, A is Acid was produced, G is Gas was produced. H1: Escherichia coli, H2: Escherichia coli, H3: Klebsiella spp., H4: Enterococcus spp., H5: Escherichia coli, H6: Bacillus spp., H7: Staphylococcus aureus.

Distribution of bacteria isolated from the urine samples of the pregnant women

Table 3 showed the distribution of *Enterococcus* spp., *Bacillus* spp. and *Staphylococcus aureus*. The most frequent isolate was *Escherichia coli* with 55% while the least frequent was *Klebsiella* spp. with 2%.

Antibiotics susceptibility test of bacterial isolates from urine of pregnant women before plasmid curing

Table 4 showed the antibiotic susceptibility of bacterial isolates from urine of pregnant women before plasmid curing. Among the Gram-positive microorganisms, *Enterococus spp.* and *Staphylococcus aureus* has the highest MRI of 1.0. *E. coli* was the most resistant bacteria among the Gram-negative microorganisms with MRI of 1.0 MRI and *Bacillus* was the least resistant with MRI of 0.8 MRI.

Table 3: Bacteria Distribution

Bacteria Isolates	Distribution (%)
Escherichia coli	55%
Klebsiella spp.	2%
Enterococcus spp.	31%
Bacillus spp.	6%
Staphylococcus aureus	6%

Table 4: Antibiotics Resistance Pattern of Bacterial Isolates from Urine of Participants before Plasmid Curing

Samples	CN	APX	Z	AM	R	CPX	S	SXT	E	PEF	MRI
G+ve											
Enterococus spp.	R	R	R	R	R	R	R	R	R	R	1.0
Bacillus spp.	R	R	R	R	R	S	R	S	R	R	0.8
Staphylococcus aureus	R	R	R	R	R	R	R	R	R	R	1.0
G-ve	CN	PEF	OFX	AZ	LEV	CF	SP	CPX	AM	AU	MRI
Escherichia coli	R	R	R	R	R	R	R	R	R	R	1.0
Escherichia coli	R	R	R	R	R	R	R	S	R	R	0.9
Klebsiella spp.	R	R	R	R	R	R	R	S	R	R	0.9
Escherichia coli	R	R	R	R	R	R	R	R	R	R	1.0

Key: (CN- Gentamycin, APX- Ampiclox, Z- Zinnacef, AM- Amoxacillin, R- Rocephin, CPX- Ciprofloxacin, S- Streptomycin, SXT- Septrin, E- Erythromycin, PEF- Pefolxacin, OFX- Ofloxacin, AZ- Azithromycin, LEV- Levofloxacin, CF- Cefotaxim, SP- Sparfloxacin and AU- Augmentin).

Plasmid profile of bacterial isolates using Agarose Gel Electrophoresis

Figure 1 showed plasmid profile of the bacteria isolates using Agarose Gel Electrophoresis.

Antibiotics susceptibility test of bacterial isolates from urine of pregnant women after plasmid curing

Table 5 showed antibiotics susceptibility of bacterial isolates from urine of pregnant women after plasmid curing. Among the Gram-positive bacteria, *Enterococcus spp*. has the highest MRI while *Bacillus spp*. has the least. Among the Gram-negative bacteria, *E. coli* has the highest MRI.

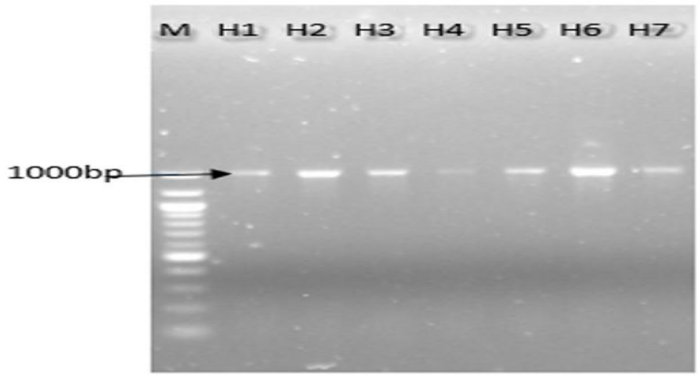

Figure 1: Agarose Gel Electrophoresis of Plasmid Profile of Bacterial Isolates.
Lane M = molecular size marker, H1: *Escherichia coli*, H2: *Escherichia coli,* H3: *Klebsiella spp*., H4: *Enterococcus spp*., H5: *Escherichia coli*, H6: *Bacillus spp*., H7: *Staphylococcus aureus.*

Table 5: Antibiotics Resistance Pattern of Bacterial Isolates from Urine of Participants after Plasmid Curing

Samples	CN	APX	Z	AM	R	CPX	S	SXT	E	PEF	MRI
G+ve											
Enterococcus spp.	R	R	R	R	R	R	R	R	R	R	1.0
Bacillus spp.	S	S	S	S	S	S	S	S	S	S	0
Staphylococcus aureus	S	R	S	S	S	S	S	S	S	S	0.1
G-ve	CN	PEF	OFX	AZ	LEV	CF	SP	CPX	AM	AU	MRI
Escherichia coli	R	R	R	S	S	R	S	R	R	R	0.7
Escherichia coli	S	R	R	R	R	R	R	R	R	R	0.9
Klebsiella spp.	R	R	R	R	S	R	S	R	R	R	0.8
Escherichia coli	S	R	R	S	R	R	R	S	S	R	0.6

Key; (CN- Gentamycin, APX- Ampiclox, Z- Zinnacef, AM- Amoxacillin, R- Rocephin, CPX- Ciprofloxacin, S- Streptomycin, SXT- Septrin, E- Erythromycin, PEF- Pefolxacin, OFX- Ofloxacin, AZ- Azithromycin, LEV- Levofloxacin, CF- Cefotaxim, SP- Sparfloxacin and AU- Augmentin). Resistance of some bacterial isolate's resistance was extra-chromosomal.

Agarose Gel Electrophoresis Analysis of beta hemolysin gene (hlb) in bacterial isolates

Figure 2 showed Agarose Gel Electrophoresis analysis of beta hemolysin gene (*hlb*) in bacterial isolates. Hemolysin gene was detected in *Escherichia coli, Enterococcus* spp., and *Staphylococcus aureus*.

Agarose Gel Electrophoresis Analysis of aph(2")-If gene of Gram-negative isolates

Figure 3 showed Agarose Gel Electrophoresis analysis of *aph(2")-If* gene of Gram-negative isolates. Resistant *aph(2")-If* gene was detected in *Escherichia coli, Enterococcus* spp., *Bacillus* spp. and *Staphylococcus aureus*.

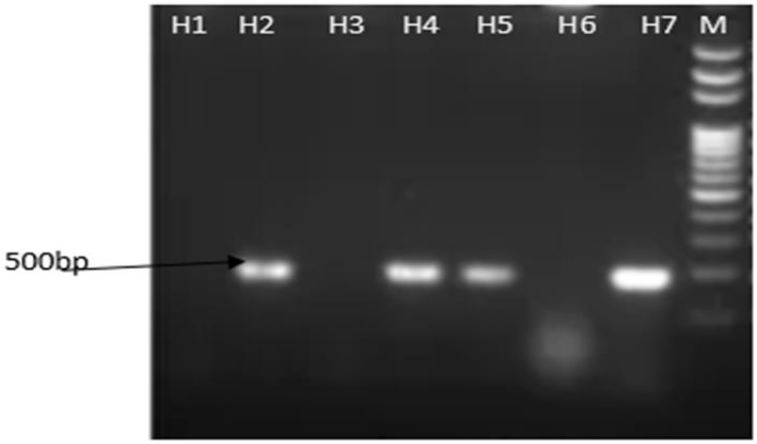

Figure 2: Agarose Gel Electrophoresis of Beta Hemolysin gene (*hlb*) in Bacterial Isolates.

M: molecular weight marker, H1: *Escherichia coli*, H2: *Escherichia coli,* H3: *Klebsiella spp*., H4: *Enterococcus spp*., H5: *Escherichia coli*, H6: *Bacillus spp*., H7: *Staphylococcus aureus.*

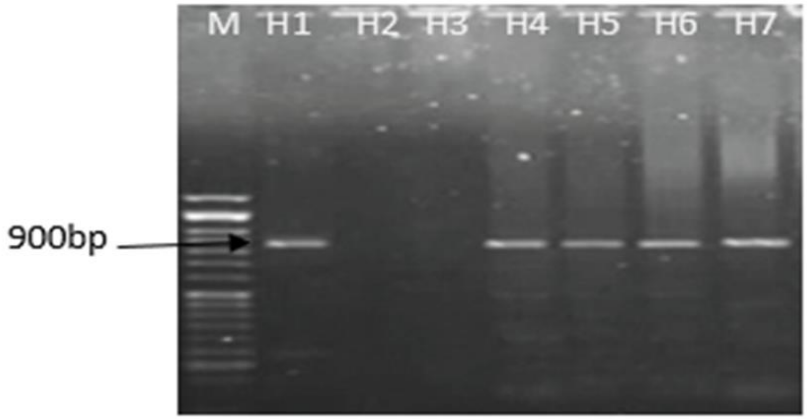

Figure 3: Agarose Gel Electrophoresis of *aph(2")-If* gene of Gram -ve isolates.

M: molecular weight marker, H1: *Escherichia coli*, H2: *Escherichia coli,* H3: *Klebsiella spp*., H4: *Enterococcus spp*., H5: *Escherichia coli*, H6: *Bacillus spp*., H7: *Staphylococcus aureus.*

DISCUSSION

In view of the escalating threat orchestrated by multi-resistant drug microbes, the study was designed to evaluate. The aim of the research was to investigate the presence of multi-drug-resistant bacteria in the urine samples of pregnant women who were in second and third trimesters and aged 10-40years attending antenatal clinics in Auchi, a sub-urban area in South-Southern region of

Nigeria. Urine sample analyzed in the study yielded significant bacterial growth. This might be due to lifestyle and water intake habit. A previous study found that 63% of pregnant women with UTI were between 18 and 25 years due to hormonal fluctuations and increased sexual activity (Rajshekher et al., 2024). In the present study, it was discovered that pregnant women aged 21 to 30 years exhibited a bacterial count of 31.57×10^3cfu/mL while those who were between 10 and 20 years has 8.00×10^3 cfu/mL.

As far as prevalence of bacteria during pregnancy was concerned, a study found that the highest rate of asymptomatic bacteriuria occurred in the third trimester (Sheppard et al.,2023). Investigation by Agarwal et al., (2021) found highest

prevalence of asymptomatic bacteriuria in the second trimester. In the present study, it was also shown that pregnant women in their third semester showed no significant difference from those who were in second trimester.

Concerning the role of educational status on infection rate during pregnant, about 69% of the participant in the present study had attained tertiary education level. However, there was no significant difference in bacteria counts of those with tertiary and secondary educational levels. This was quite intriguing and it disagreed with an Iranian study which reported that higher educational levels were associated with improved health literacy and self-efficacy leading to better UTI preventive behaviors (Elsami et al., 2023). However, medical negligence and personal hygiene might be responsible for the trend observed in the present study (Ali et al. ,2021). The roles of personal hygiene in infection prevention cannot be underestimated. A study conducted among Jordanian women found that those who changed their underwear less frequently were at a higher risk of developing UTIs (Hatemleh et al.,2024).

It was observed that about 48.3% of participant with history of urinary tract infection showed significant presence of bacteria. There was significant difference in bacteria count of participants with no history of urinary tract infection and those who reported urinary tract infection history. In fact, lower counts were observed in those who had no history of infection. This implies that the prior acquisition of infection plays key determining role in urinary tract infections during conceptions.

Among the isolates, *Escherichia coli* was the most prevalent. Most of the significant bacteria count of 72.4% came from participant between 21-30 years based on the history of urinary tract infection. A study conducted at Corniche hospital found that a prior UTI was significantly associated with an increased risk of UTI in pregnancy (Balachandran et al., 2022). However, even with no history, hormonal changes during pregnancy as well as mechanical compression and altered immunity can increase risk of urinary tract infection.

Furthermore, a total of 7 isolates were identified after the cultural, morphological and biochemical characteristics tests were carried out. *Escherichia coli* was found to be circular, yellow in color, small in size, raised elevation, entire margin and opaque in transparency. It was confirmed through Gram stain to be a Gram-negative single rod. *E. coli* was found to be indole positive, citrate negative, catalase positive, oxidase negative, urease and H_2 S negative. It was confirmed of

the ability to ferment glucose, lactose and sucrose all while producing gas. All of these results were compiled and confirmed by standard references. *Klebsiella spp*. was also identified after compilation and confirmation of results. *Enterococcus spp., Bacillus spp.,* and *Staphylococcus aureus* were also identified. Antibiotics sensitivity was carried out to check for resistance in these organisms that have been identified. Of the 7 isolates, 3 were positive while 4 were negative. The Gram-positive isolates exhibited high level of resistance with *Enterococcus spp.* and *Staphylococcus aureus* isolates exhibiting resistance. Sequel to plasmid curing, the removal of plasmid from the isolates to find out if the resistance was extra chromosomal, another antibiotics sensitivity test carried out indicated that *E. coli* and *Klebsiella spp.,* isolates still exhibited high level of multi drug resistance.

Enterococcus spp. isolate exhibited drug resistance while the remaining *Bacillus spp.* and *Staphylococcus aureus* isolates were no longer resistance as they were sensitive to all antibiotics. This showed that only 2 of the isolates resistance were extra chromosomal. The Gram-negative isolates also had *E. coli* and *Klebsiella spp.* isolates exhibiting high level of multi drug resistance and two *E. coli* isolates exhibiting pan drug resistance. This poses a serious health issues for pregnant women. This test showed that all isolates carries an extra chromosomal DNA (plasmid) at 100bp. *Staphylococcus aureus* exhibited the beta hemolysin gene virulence factor at 500bp with thick bands while *E. coli, Klebsiella spp*. and *Bacillus spp* had no beta hemolysin gene, a cause of complete lysis of red blood cells. Resistant genes occurred at 900bp with moderate bands using aph(2")-If gene for Gram negative because most of the isolates are Gram negative. The resistance may not really be dependent on plasmid. All isolates except one *E. coli* and *Klebsiella spp*. had resistance gene. Only *E. coli*, H1 had resistant gene. This may be because the rest were of different strains.

CONCLUSION

The study revealed a high prevalence of multidrug-resistant bacteria in the urine of pregnant women during the second and third trimesters. There is urgent need for mandatory routine microbial screenings during antenatal care.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Edo State University, Iyamho for providing laboratory facilities and equipment, Hope Hospital and Academic supervisor; Dr. (Ms) Phebean O. Ozolua.

CONFLICT OF INTEREST

None declared.

AUTHORS' CONTRIBUTIONS

1. Ozolua, P.O contributed to the Conceptualization, study design, data collection and analysis.
2. Adeniyi, M.J generated the manuscript draft.
3. Onize, K.H coordinated data collection.
4. Evbuomwan, L contributed to data collection.

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