Prevalence and Antibiogram of ESBL-positive Uropathogenic *Escherichia coli* in Pregnant Women in Onitsha Metropolis, Nigeria

Amala Stellamaris Emekekwue[1], Malachy Chigozie Ugwu[1]*, Chika Peter Ejikeugwu[2,3], David Chinemerem Nwobodo[1,4], Nonye Treasure Ujam[1,3], Ugochukwu Moses Okezie[1], Ifeanyichukwu Romanus Iroha[2], Charles Okechukwu Esimone[1]

[1]Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University Awka, Nigeria.
[2]Department of Applied Microbiology, Ebonyi State University Abakilikii, Nigeria.
[3]Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, ESUT Nigeria.
[4]Department of Microbiology, Renaissance University, Enugu, Nigeria.

ABSTRACT

The study investigated the frequency and antimicrobial susceptibility profiles of uropathogenic *E. coli* from pregnant women. Three hundred and thirty (330) mid-stream urine samples from pregnant women attending 2 antenatal clinics in the Onitsha metropolis were bacteriologically analyzed for the selective isolation of *E. coli* using standard techniques. Antibiotic susceptibility testing was carried out using the modified Kirby-Bauer disk diffusion technique. ESBL production was phenotypically detected using combined disk diffusion techniques, and confirmed using PCR technique. Of the 330 urine samples, 102 (30.9%) *E. coli* isolates were isolated. The prevalence of uropathogenic *E. coli* among the study volunteers was highest in the age group 26–35 years (37.9%), followed by those in 3rd trimesters (35.6%). A significant frequency of *E. coli* was recovered from business women (39.4%), multiparous (48.6%), and those with a history of UTI infection (48%). Antibiotic susceptibility tests showed that the most effective antibiotics against the strains were ofloxacin (58.8%) > gentamicin (57.8%) > nitrofurantoin (54%) > ciprofloxacin (51%) and cefixime (49%). Fifty-three (52%) isolates were multidrug resistant whereas 66.7% had a multiple antibiotics resistance index of > 0.2. Sixty-nine (67.6%) isolates were potential ESBL producers while 21(30.4%) isolates were confirmed ESBL producers. PCR results revealed that the uropathogenic*E. coli* harbored the ^{bla}TEM (66.7%) and ^{bla}SHV (38.1%) genes. A 30.9% prevalence of uropathogenic*E. coli* was detected and was significantly associated with the participant's age, gestation, occupation, education level, UTI history, and parity at *P-value* < 0.05. ^{bla}TEM was the most predominant ESBL gene detected.

Key Words: *Uropathogens, Escherichia coli, ESBL, AmpC, Pregnant women, Antibiotic resistance*

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INTRODUCTION

Urinary Tract Infection (UTI) is an inflammatory response to the invasion of microorganisms in the urothelium [1-3]. They are bacterial infections that affect parts of the urinary tract system, and are widespread in humans, with higher frequency in women due to the closeness of their urethra to the anus and hormonal changes. UTI is diagnosed when the number of microorganisms detected in mid-stream clean catch urine is $\geq 10^5$ cells per milliliter [1, 4-6]. UTI is treatable, however, it is currently becoming more difficult to control because of antibiotic resistance [4, 7, 8].

The incidence of antibiotic resistance among bacteria and

Corresponding author: Malachy Chigozie Ugwu
Address: Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University Awka, Nigeria.
E-mail: ✉ mc.ugwu@unizik.edu.ng
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its effects cannot be over-emphasized. It is progressively on the increase as decades pass and a lot of bacteria have acquired great resistance to more antibiotics [9]. Antibiotic resistance has become a great threat to global public health both in hospitals and communities with the major cause of resistance to some multidrug resistance factors including extended-spectrum beta-lactamases (ESBL) and AmpC beta-lactamases detected in clinical laboratories. These are mostly detected in Gram-negative bacteria from the *Enterobacteriaceae* family with *Escherichia coli* as one of the most common organisms involved in expressing these resistance traits [10].

ESBL resistance implicated in urinary tract infections has emerged as an important cause of resistance among Gram-negative bacteria [4]. The alarming trend is the maternal colonization of these organisms during pregnancy [11]. With the physiological changes caused by gestation, pregnant women are prone to bacterial infections. There have been worldwide reports of ESBL colonization during pregnancy [12-14] and are steadily increasing in Nigerian communities [15-17]. ESBL-producing microorganisms express resistance to all penicillins (except temocillin), cephalosporins (except cefoxitin and cefotetan), and aztreonam. However, they can be inhibited by clavulanic acid, which is a beta-lactamase inhibitor. Hence, few treatment options are available for infections caused by organisms producing ESBLs. This is life-threatening and specifically dangerous in pregnant women because they are being exposed to risks of severe health challenges as a result of hormonal/ physiological changes. UTIs are very common in women and more so during gestation. Half of those with asymptomatic bacteriuria later develop pyelonephritis and experience higher incidences of intrauterine growth restrictions and low birth weight infants [18]. The occurrence of UTI in awaiting mothers reportedly promotes risks of preterm labor, preterm birth, pregnancy-induced hypertension, abortion, cesarean deliveries, pre-eclampsia, amnionitis, and anemia [17].

The AmpC β-lactamase enzyme hydrolyzes all beta-lactam antibiotics except cefepime and carbapenems [10, 19]. They are less commonly detected and can be encoded in bacterial chromosomes as well as in plasmids. AmpC-producing organisms have a high ability to transfer drug-resistance features to other bacteria through genetic elements such as plasmids [20]. As a result, therapeutic options are limited. Clavulanic acid and other inhibitor-based combinations do not inhibit the AmpC enzymes and that differentiates them from the ESBLs [21].

The rate of resistance of community-acquired uropathogens has not been studied extensively in Onitsha, particularly in uropathogens from pregnant women. To date, there is a dearth of data on bacterial resistance among pregnant women with UTIs in the Onitsha metropolis. Hence, this study was undertaken to investigate the prevalence rate, antimicrobial susceptibility profile and the prevalence of ESBLs/AmpC phenotypes among uropathogenic *E. coli* isolates from antenatal care mothers in the Onitsha metropolis.

MATERIALS AND METHODS

Study area, population, and sample size

Samples were collected at antenatal clinics of two General Hospitals within the Onitsha metropolis in Anambra State, Nigeria. Oral and written consent was obtained from the study participants, after which they were educated on how to obtain and transport the clinical samples (mid-stream urine) to the laboratory for further analysis. The Local Ethics and Research Committee of Nnamdi Azikiwe University, Awka provided ethical approval for this study (Ref: SHMB/AD.196/VOL.IV/138). This is a descriptive cross-sectional study involving 330 pregnant women attending ante-natal clinics. It was conducted from February to December 2020. The prevalence rate of ESBL-producing *Escherichia coli* inhabiting pregnant women in Nnewi, Anambra State, is 78.1% [17].

Preparation of proforma and data collection

To better understand the socioeconomic factors contributing to the evolution and spread of antibiotic-resistant bacteria amongst pregnant women in Onitsha, Anambra State of Nigeria, questionnaires/proformas were designed and used to obtain information about antibiotic resistance. A well-structured questionnaire was used as a data collection instrument. It was developed to understand the women's age, education, occupation, gestation age, and history of urinary tract infections - to understand how they relate to antibiotic resistance. The questionnaire was reviewed by five researchers, experts, medical practitioners, and the ethical committee of both hospitals included in the study. It was pre-tested on twenty pregnant women from other hospitals and adjustments were made to validate its content. The study participants were recruited by the researcher with the help of nurses on duty. The researcher visited the clinic during ante-natal sessions. The selection criteria involved women with complaints of any UTI symptoms who have not been on any antibiotic treatment for two weeks before the survey. The participants were briefed on the reason for the research by the physician on duty as well as the researcher. Emphasis was laid on voluntary participation, also the unanimous and confidentiality of their responses was assured. Questionnaires were assigned numbers

identical to the labeling on the urine bottles. Care was taken to ensure no duplication.

Collection and processing of samples

Each participant collected mid-stream clean catch urine samples into wide-opened sterile universal bottles after being properly instructed on the procedure of collection without touching the skin. The urine samples were labeled appropriately and transported within two hours of collection in iced packs carrier to the Microbiology Laboratory Unit of NnamdiAzikiwe University, Agulu campus. To detect significant bacteriuria, a semi-quantitative culture method was used. Using an appropriate loop, culture was done on blood and Mac-Conkeyagar plates. All samples were analyzed bacteriologically within two hours of collection. Each collected sample was inoculated into 5 ml of freshly prepared nutrient broth. The tubes were loosely covered with cotton wool and incubated for 18- 24 hrs at 37℃. Growth of bacteria was identified by cloudiness or turbidity in the broth culture after incubation, and bacteriuria was inferred when there were at least 10^5 organisms/ml of urine [22].

Culture and characterization

The turbid solution from the overnight broth culture was inoculated aseptically on MacConkey agar (MAC) plates and incubated at 37°C for 18-24 hours. Suspect colonies of *Escherichia coli* species were subcultured onto freshly prepared MacConkey and nutrient agar plates for the isolation of pure cultures of *E. coli* species. The isolates were further identified based on their colonial, biochemical, microscopic, and morphological characteristics using citrate test, indole test, and microscopy (Gram staining) [23].

Antibiotic susceptibility studies

Antimicrobial susceptibility studies were done using the modified Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) protocol. Pure cultures of identified bacteria were adjusted to 0.5 MacFarland turbidity standard [24]. In this method, lawn cultures of the bacteria were made on Muller-Hinton agar plates using sterile swab sticks, then antibiotic disks were aseptically placed on the media using sterile forceps and gently pressed to ensure even contact with the media. ABTEK Gram-negative disks of ceftazidime (30ug), cefuroxime (30ug), gentamicin (10ug), cefixime (5ug), ofloxacin (5ug), amoxicillin-clavulanic (30ug), nitrofurantoin (300ug) and ciprofloxacin (5ug) were used. The plates were incubated for 18-24hrs at 37°C and the IZD produced were measured and interpreted based on CLSI breakpoints [25].

Determination of the multiple antibiotic resistance index (MARI)

The multiple antibiotic resistance indexes of the isolated *E. coli* isolates were calculated using the formula; MARI=a/b; where 'a' is the number of antibiotics thatthe resistant bacteria wereresistant to, and 'b' represents the total number of antibiotics which the resistant bacteria has been evaluated for.

Screening isolates for AmpC enzymes

The test is based on the ability of tris-EDTA to permeate bacterial cells and release β-lactamases into the external environment. A lawn culture of cefoxitin-susceptible *E. coli* previously standardized to 0.5 MacFarland turbidity standards was made on a Mueller-Hinton agar plate. A 30 µg cefoxitin disc was placed gently on the agar surface. Then, a sterile plain disc (6mm) was moistened with sterile saline (20µl) and aseptically inoculated with several colonies of the test organism. The inoculated disc was then placed beside the cefoxitin disc on the inoculated plate and then incubated overnight at 37°C. The plates were scrutinized for flattening of the zone of inhibition by cefoxitin in the vicinity of the test disc, indicating enzymatic inactivation of cefoxitin (positive result) or the absence of distortion, indicating no significant inactivation of cefoxitin (negative result) [26].

ESBL screening

ESBL producers were also detected by the disk diffusion test. All isolates that were initially resistant to at least one of the third-generation cephalosporins (Ceftazidime or cefixime) by antibiotic susceptibility tests were considered potential ESBL producers as recommended by the CLSI and further tested by confirmatory methods. Phenotypic confirmation of ESBL was done by the double-disc synergy test (DDST). All the potential ESBL producers were subjected to the confirmatory procedure of DDST on Mueller-Hinton-agar plates using a disc of amoxicillin-clavulanate (20/10µg) together with two cephalosporins, cefotaxime, and ceftazidime (Oxoid, UK). 0.5 McFarland turbidity standards of the test isolates were swabbed on Mueller-Hinton agar plates using sterile swab sticks as recommended by CLSI [24]. The disc containing amoxicillin-clavulanate (20/10 µg) was placed in the center of the plates while the two 3rd generation cephalosporins were placed 30mm apart respectively and 15mm center to center of the amoxicillin-clavulanate disc. After incubation at 37°C for 18-24hrs, confirmation of ESBL producing organism was determined when the zone of inhibition around ceftazidime (CAZ) and cefotaxime

(CTX) disks expanded by at least 5 mm close to amoxicillin-clavulanate (AMC) disk.

DNA extraction

Isolation of the genomic DNA was by boiling method; Seventy- two hour-broth cultures of the organisms were centrifuged briefly at 12000 revolutions per minute for 5 minutes at 4ºC. Cell pellets were harvested in Eppendorf tubes and washed and about 50µl of nuclease-free water was added to the residual pellets. The resulting solution was subjected to a heat shock in a water bath at 99ºC for 10 minutes and then to ice shock treatment for 30 mins. Subsequently, the solution was centrifuged at 12000rpm for 10 mins at 4ºC after which 50 µl of the supernatant was transferred to another Eppendorf tube and stored at 4ºC until further use.

PCR amplification of ESBL and AmpC resistance genes

The isolated DNA of all the phenotypically confirmed ESBL-positive isolates wasfurther analyzed for ESBL and AmpC genes using the PCR technique. To detect these genes, three primer pairs (forward and reverse) for screening the blaTEM, blaSHV, and blaCTX-M genes and a primer pair for detecting the AmpC gene were used in the PCR reaction. The primer sequences are shown in **Table 1**.

Table 1. The Primer Sequences of the ESBL and AmpC Genes Used

Phenotypic resistance	Target gene	Primer name	PRIMER SEQUENCE $5^1 \rightarrow 3^1$	Amplicon size (base pair)	Annealing Temp.	References
ESBL	CTX-M	Forward (F)	GACAAAGAGAGTGCAACGGATG	501	56^0C	[27]
	CTX-M	Reverse (R)	TCAGTGCGATCCAGACGAAA			,,
	TEM	Forward (F)	AGTGCTGCCATAACCATGAGTG	431	56^0C	[27]
		Reverse (R)	CTGACTCCCCGTCGTGTAGATA			,,
	SHV	Forward (F)	GATGAACGCTTTCCCATGATG	214	56^0C	[27]
		Reverse (R)	CGCTGTTATCGCTCATGGTAA			,,
AmpC		Forward (F)	GGTATGGCTGTGGGTGTTA	882	56^0C	[28]
		Reverse (R)	TCCGAAACGGTTAGTTGAG			,,

The Polymerase chain reaction was carried out using the Solis Biodyne 5X FIREPol Blend Master mix. It was performed in a total volume of 25 µl of a reaction mixture. The reaction concentration was diluted to 1X concentration containing 1X Blend Master mix buffer (Solis Biodyne), 1.5 mm $MgCl_2$, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 20pMol of each primer (Jena Bioscience, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), proofreading enzyme and extracted DNA (5µl). Sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted using a Techne thermal cycler (3' Prime Model). Initial denaturation was at 95°C for 5 minutes followed by 35 amplification cycles for 30 seconds at 95°C; 40 seconds at 56°C (for ESBL) and 2 minutes at 72°C. This was succeeded by a final extension step of 10 minutes at 72°C.

Agarose gel electrophoresis

The PCR amplification products were separated on a 1.5% agarose gel. Electrophoresis was carried out at 80V for 1 hour and 30 minutes. A 100 base pairs DNA ladder (Solis Biodyne) was used as the DNA molecular weight marker. At the end of electrophoresis, DNA bands were visualized by ethidium bromide staining, and the detected genes were photographed in an ultraviolet transilluminator.

Statistical analysis

The results obtained were analyzed statistically using SPSS version 16 software to determine the significance of the study at a 95% confidence interval. Firstly, a descriptive analysis was done on the distribution of isolates according to the socio-demographic characteristics and clinical history of the volunteers and the statistics were expressed in numbers and percentages. Further analysis involved using the Chi-squared test to establish significant associations between the socio-demographic characteristics and *E. coli* colonization or non-colonization. *P* values less than 0.05 were considered statistically significant, thus we reject the Null Hypothesis which states that mid-stream clean catch urine samples of pregnant women do not harbor uropathogenic *E. coli* that are multidrug-resistant as well as ESBL/AmpC producers, and accept Alternate Hypothesis which states that mid-stream clean catch urine samples of pregnant women do not harbor uropathogenic *E. coli* that are multidrug-resistant as well as ESBL/AmpC producers.

RESULTS AND DISCUSSION

Prevalence of E. coli isolates from the urine of pregnant women

A total of 102 (30.9%) isolates of *Escherichia coli* were identified by colonial/cultural morphology and biochemical tests from the urine samples of 330 pregnant women recruited and bacteriologically investigated in this study.

The distribution of the *E. coli* isolates among the pregnant women according to their Age Brackets is shown in **Table 2**.

The highest prevalence of *E. coli* occurred in pregnant women of the age group between 26-35 years. The total number of samples in this age group was 145, and 55 (37.9%) were colonized with *E. coli*, - followed by the age group 16-25 years (33.3%) - and the age group 36-45 years which had the lowest prevalence of 15%.

According to the gestation period, pregnant women in their 3rd trimester had the highest prevalence of *E. coli* (35.6%) while those in their 2nd trimester had a prevalence of 29.5%, and those in their 1st trimester had the least prevalence rate of 18.2%.

According to the level of education, the highest percentage occurrence was seen in pregnant women with primary education only (50%), followed by those at the secondary education level (37.5%) whereas those with tertiary education had the lowest prevalence rate (26%). Education may have played a role in the level of hygiene of the pregnant women investigated in this study since those with higher education had a lower prevalence of *E. coli* than their counterparts with a lesser education and who may not have been well acquainted with some personal hygiene protocols to keep such microbes at bay, especially during pregnancy.

According to occupation, pregnant women who are business women had the highest percentage of occurrence (39.4%), followed by students (28.2%) and house wives (25%) while civil servants had the least percentage distribution (17.5%).

According to parity, the highest percentage occurrence was seen in pregnant women who have had multiple deliveries (48.6%) while those who are primiparous had the least percentage distribution (21%).

Women with a history of UTI also had a higher prevalence (48%) than those without a history of UTI (20.5%). This could be attributable to re-infection or failure of treatment due to resistance of the uropathogens which could not be extinguished by available antibiotics.

Table 2. Distribution of *E. coli* isolates among pregnant women according to the demographic data

Demographics/ Clinical data	Group	No of samples tested	No with *E. coli* (%)	No without *E. coli* (%)	X^2	*P* value
Age brackets	16-25 years	105	35 (33.3%)	70(66.7%)	13.12	0.001415
	26-35 yrs	145	55 (37.9%)	90(62.1%)		
	36-45 yrs	80	12 (15%)	68(85%)		
Gestation age	1st Trimester	55	10(18.2%)	45(81.8%)	6.08	0.047806
	2nd Trimester	95	28(29.5%)	67(70.5%)		
	3rd Trimester	180	64(35.6%)	116(64.4%)		
Level of education	Primary	10	5(50%)	5(50%)	6.41	0.040602
	Secondary	120	45(37.5%)	75(62.5%)		
	Tertiary	200	52(26%)	148(74%)		
Occupation	Student	25	11(44%)	14(56%)	17.99	0.000441
	Housewife	20	5(25%)	15(75%)		
	Civil servant	120	21(17.5%)	99(82.5%)		
	Businesswomen	165	65(39.4%)	100(60.6%)		
Parity	Nulliparous	125	30(24%)	95(76%)	22.74	1.15E-05
	Primiparous	100	21(21%)	79(79%)		
	Multiparous	105	51(48.6%)	54(51.4%)		
UTI history	UTI History	125	60(48%)	65(52%)	27.51	1.56042E-07
	No UTI History	205	42(20.5%)	163(79.5%)		

Antibiotic susceptibility pattern of the isolated uropathogenic E. coli

The isolated *E. coli* organisms were tested against eight different antibiotics (ABTEK Gram-negative multi-disk). The antimicrobial susceptibility pattern revealed varied levels of sensitivity and resistance of the *E. coli* isolates to the tested antibiotics **(Table 3)**. The results showed remarkable resistance to amoxicillin-clavulanic (55.9%), cefixime (47.1%), cefuroxime (49%), and ceftazidime (41.2%) whereas high sensitivity was recorded with gentamicin (60.8%), ciprofloxacin (59.8%) ofloxacin (58.8%), nitrofurantoin (51%), and ceftazidime (44.1%).

Of the 102 *E. coli* isolates identified in this study, 53(52%) isolates were found resistant to three or more different classes of antibiotics and hence termed multi-drug resistant **(Figure 1)**.

The MARI profile of the 102 *E. coli* isolates is shown in **Table 4**. All the isolates were resistant to at least one antibiotic. Most of the isolates 68(66.7%) were MAR isolates with MAR indices greater than 0.2 while those less than 0.2 were 34(33.3%). The commonest MAR index was 0.1(33.3%) while the calculated average MARI of the 102 isolates was 0.4. This implies that the resistant *E. coli* isolates recovered from the urine samples are multidrug-resistant and shows resistance to antibiotics in at least four different classes.

Table 3. Antimicrobial susceptibility of 102 strains of *E. coli*

Antibiotic Tested	Resistance		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Ceftazidime(30ug)	42	41.2	15	14.7	45	44.1
Cefuroxime(30ug)	50	49	21	20.6	31	30.4
Gentamicin(10ug)	26	25.5	14	13.7	62	60.8
Cefixime(5ug)	48	47.1	15	14.7	39	38.2
Ofloxacin(5ug)	30	29.4	12	11.8	60	58.8
Amoxicillin-clavulanic (30ug)	57	55.9	20	19.6	25	24.5
Nitrofurantoin(300ug)	34	33.3	16	15.7	52	51
Ciprofloxacin(5ug)	27	26.4	14	13.7	61	59.8

Table 4. Distribution of Multiple Antibiotic Resistant Index of the isolates

MARI	NO	%
0.1	34	33.3
0.3	10	9.8
0.4	24	23.5
0.5	15	14.7
0.6	8	7.8
0.8	2	2
0.9	1	1
1.0	8	7.8

Phenotypic detection of AmpC and ESBL-positive isolates

Out of the 102 *E. coli* isolates phenotypically tested for AmpC phenotypes, none was positive for AmpC enzyme production as no distortion or flattening of the zone was formed

Of the 102 *E. coli* isolates, 69(67.6%) were resistant to one or two third-generation cephalosporins by the preliminary screening test and thus inferred to be potential ESBL producers. However, 21(30.4%) isolates of *E. coli* were phenotypically confirmed as ESBL producers by the Double Disk Synergy Test (DDST) method **(Table 5)**.

Table 5. Distribution of ESBL Positive isolates

No of Samples	No of *E. coli* isolates n(%)	Potential ESBL producers n(%)	AmpC producers n(%)	Confirmed ESBL producers by DDST n(%)
330	102(30.9%)	69(67.6%)	0(0%)	21(30.4%)

Genotypic detection

PCR was performed on the 21 confirmed ESBL-producing isolates and ESBL genes were detected in 14 *E. coli* isolates. The gel electrophoresis results revealed ^{bla}TEM (66.7%) and ^{bla}SHV (38.1%) genes and no amplification for ^{bla}CTX-M and AmpC genes were detected **(Table 6, Figure 1)**. Some of the isolates have both genes present while some have one gene present. Eight isolates had both genes present. However, no isolate

had ^{bla}SHV genes only while 6 isolates had ^{bla}TEM genes only.

The distribution of ESBL genes according to the demographic and clinical data of the volunteers is presented in **Table 7**.

Table 6. Percentage occurrence of detected genes

ESBL genes	No of Occurrence	%
CTX	0	0
TEM	14	66.7
SHV	8	38.1
AmpC genes	0	0

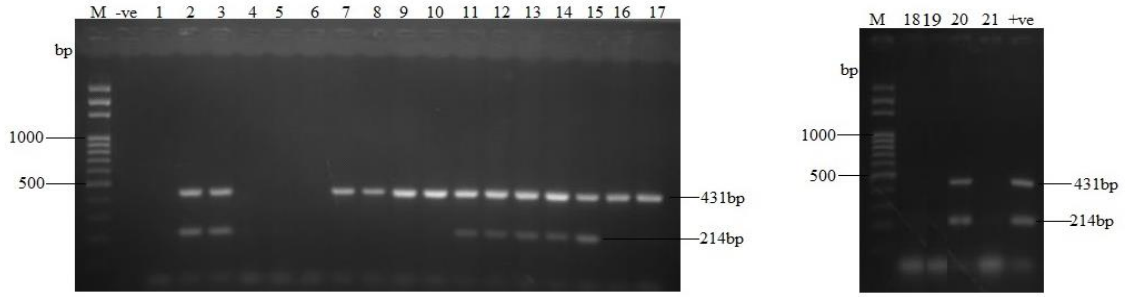

Figure 1. Gel electrophoresis photograph revealing the amplified DNA bands

Table 7. Distribution of ESBL genes according to the Demographic and Clinical data of the pregnant women

Demographics/Clinical data	Group	ESBL genes detected
Age	16-25 years	3 TEM+ 3 SHV
	26-35 yrs	8 TEM + 4 SHV
	36-45 yrs	3 TEM + 1 SHV
Gestation age	1st Trimester	3 TEM + 2 SHV
	2nd Trimester	6 TEM + 3 SHV
	3rd Trimester	5 TEM + 3 SHV
Level of education	Primary	6 TEM + 3 SHV
	Secondary	3 TEM + 2 SHV
	Tertiary	5 TEM + 3 SHV
Occupation	Student	4 TEM +1 SHV
	Housewife	2 TEM + 2 SHV
	Civil servant	3 TEM + 2 SHV
	Businesswomen	5 TEM + 3SHV
Parity	Nulliparous	3 TEM + 2 SHV
	Primiparous	5 TEM + 2 SHV
	Multiparous	6 TEM + 4 SHV
UTI history	UTI History	9 TEM + 5 SHV
	No UTI History	5 TEM + 3 SHV

Uropathogenic *E. coli* (UPEC) is one of the highest reported causes of UTI, usually resistant to multiple antibiotics and resulting in recurrent UTIs, mostly among the high-risk population [2]. An increase in multidrug resistance in bacterial uropathogens is an evolving public health challenge. Antimicrobial resistance has become a global threat facing many countries and continents. Developing countries like Nigeria are not an exemption. This has been attributed to empirical antibiotic therapy, inappropriate prescription, and misuse of antibiotics [29]. These factors significantly contributed to the evolution of drug-resistant pathogens and their adverse spread in both hospitals and non-hospital environments.

Pregnancy brings numerous hormonal and physiological changes in women's bodies that tend to increase the likelihood of urinary tract infections (UTIs). These changes, along with an already short urethra and improper hygiene due to a protruded (pregnant) belly, help make UTIs the most common bacterial infection during pregnancy [1]. Complications can arise from reoccurring UTIs. When bacteriuria is left untreated during pregnancy, both the fetus and the mother are faced with big risks such as preterm birth, pyelonephritis, low birth weight, and increased perinatal mortality.

Escherichia coli have been recorded as the commonest causative agent of UTI among pregnant women. This study recorded a prevalence of 30.9% uropathogenic *E. coli* isolates. Of the 330 urine samples screened, *Escherichia coli* constituted about one-third. The result is in line with the work of Ghaima *et al.*, [30] who identified 34% uropathogenic *E. coli* from urine samples of pregnant women.

The prevalence of uropathogenic *E. coli* isolates according to their demographic characteristics and clinical history was analyzed in this study. The prevalence of uropathogenic *E. coli* amongst the study participants was significantly highest within the 26 – 35 years age group. Considering their age, they must have been the most sexually active group and so it is presumed that high sexual activities may have highly exposed them to

significant bacteriuria. According to Parveen *et al*., [31] and Amala and Nwokah, [32] the occurrence of bacteriuria in women increases with an increase in sexual activities and the use of contraceptives. The findings from this study are similar to previous reports of positive cases of bacteriuria in pregnancy among women who are between 26–35 years of age recorded byRajshekhar and Umashenkar, and Amala and Nwokah [32, 33].

History of past UTI was also reported as a risk factor for *E. coli* colonization among pregnant women in this study. Almost half of the participants who had UTIs in the past were affected. This is in line with the work of Alemu *et al.*, [34] who recorded UTIs to be significantly higher among pregnant women with a previous history of UTIs. Physiological and hormonal changes affecting the entire urinary tract during pregnancy have also been reported to promote the risks of UTI during gestation and have been reported to occur mostly in women who have pregnancies in rapid succession, though variations occur [1, 35, 36]. These studies explain the highest rates of prevalence recorded amongst pregnant women who are in their 3rd trimesters and those who have had multiple deliveries (multiparous). This is in line with the work of Thapa *et al.*, [36] who listed multiparity as a risk factor for UTI. This high rate was also recorded amongst pregnant women who are business women and in those whose highest education level is primary school. Over one-third of the isolates were implicated in business women. This may be a result of the nature of their occupation. They might not have devoted quality time to self-care. Secondly, they are more exposed to the usage of public toilets within the market. Again, illiterate women might not have good genital hygienic practices. According to Ayogu *et al.*, [37] certain behaviors contribute to the contraction of UTIs in women, for instance, wiping from back to front when cleaning the anal area after defecating, improper cleaning of the genital area after passing urine, and not washing the hands before and after using the toilet.

Previous studies reported the colonization of the urinary tract with antibiotic-resistant organisms in Nigerian patients [4, 14]. Varying rates of prevalence have been recorded by different authors and *E. coli* constitute more than half of the resistant isolates identified amongst pregnant women with a UTI. The antibiotic susceptibility studies conducted in this study revealed the isolates to be more resistant to amoxicillin-clavulanic, cefixime, cefuroxime, and ceftazidime when compared to non-beta lactam antibiotics including gentamicin, ciprofloxacin, ofloxacin, and nitrofurantoin. This high resistance could be attributed to the production of beta-lactamase enzymes that deactivate the cephalosporins and generally acquired resistance due to the over-exposure of the organisms to the drugs. The limited use of nitrofurantoin in therapy may account for its low resistance recorded. These findings suggest that antibiotics that exhibited lesser resistance (more sensitive) can be better treatment choices in UTI management. This supports the works of Thapa *et al.*, [36] and Ayogu *et al.*, [37] who reported gentamicin and nitrofurantoin as more sensitive antibiotics.

In this study, more than half of the *E. coli* isolates (52%) were multidrug resistant. This moderately high multidrug resistance may be due to many factors which include antibiotics misuse by the public and wrong or inappropriate prescriptions by healthcare professionals, non-skilled practitioners, and the general public, as well as poor regulation of antibiotic use to mention but few. This supports the work of Joseph *et al.*, [38] who recorded a high occurrence rate of multiple drug-resistant bacteria.

MAR index value greater than 0.2 indicates a high-risk contamination source where antibiotics are often used [39]. More than half of *E. coli* isolates had a MAR index > 0.2 in this study, 0.1 was the commonest while the average index was 0.4. This indicates that antibiotics are commonly used where the isolates are from. It also shows that the organisms have been previously exposed to antimicrobial agents. In other words, the contamination source is associated with a high rate of antibiotic resistance. This is not in line with the work of Joseph *et al.*, who reported more than half of the *E. coli* they isolated had a MAR index less than 0.2 [38]. However, it is similar to the study done by Ayogu *et al.*, [37] which reported an average MARI of 0.41 for isolated *E. coli* isolates.

This study also reported more than half of the isolates as potential ESBL producers, however, confirmed ESBL producers by DDST were almost one-third of isolates. The majority of the MDR strains were also noted to be ESBL producers and this explains the high resistance of the isolates to cephalosporins. This is in line with a review done by Tanko *et al.*, [40] that recorded the prevalence of ESBL-producing bacteria in south-eastern Nigeria to range from 8.1%-74.3%. It also supports the findings of Onwuezobe and Orok, [18] who reported a similar occurrence rate of ESBL-positive *E. coli*. However, their finding was a bit higher, and this can be attributed to the sample size variations. Our study recorded no production of AmpC enzymes. This contradicts the previous work of Onyekere *et al.*, [17] who identified AmpC-producing isolates from urine samples of pregnant women. Regardless, it supports the work of Ejikeugwu *et al.*, [41] who also recorded no production of AmpC enzymes from clinical samples investigated in their study.

The study also analyzed the occurrence rate (prevalence) of Extended Spectrum Beta Lactamases genes in the isolates. PCR was performed on all *E. coli* isolates showing ESBL phenotypes and ^{bla}TEM and ^{bla}SHV genes were detected with zero amplification for the ^{bla}CTX-M gene. This proves PCR is an effective and efficient tool for gene detection in antibiotic-resistant strains. The most dominant gene detected was the ^{bla}TEM gene, which has a higher prevalence than the ^{bla}SHV gene. These ESBL genes are implicated in the inactivation of the β-lactam drugs through disruption of the amide bond of the beta-lactam ring by the ESBL enzymes. As a result, beta-lactam drugs targeted at organisms having these resistant genes do not have any effect on the organisms. These genes have been reportedly implicated in UTIs among pregnant women and this exposes them to the dangers of treatment failure amidst UTI complications. Similar work done by Onyekere *et al.*, [17] in Nnewi, southeastern Nigeria revealed the ^{bla}TEM gene as the most dominant gene. Some isolates in this study harbored more than one type of b-lactamase gene and this is seen in the co-existence of the ^{bla}TEM and ^{bla}SHV genes in eight isolates. The carriage of a single gene was also detected and this was observed in six isolates having ^{bla}TEM genes only. None of the isolates had only ^{bla}SHV genes as they always coexisted with ^{bla}TEM genes in the isolates they were present in. These findings are also in support of the work of Tanko *et al.*, [40] who listed out the most dominant ESBL genes to be ^{bla}TEM followed by ^{bla}SHV genes. However, it is in contrast with the work done by Ghaddar *et al.*, who reported ^{bla}CTX-M to be the most dominant [29].

CONCLUSION

This study reported a high prevalence of UPEC strains (30.9%) in pregnant women in Onitsha, Nigeria, and these were found to be multidrug-resistant, with the majority of the strains producing ESBL and harboring the blaTEM gene that mediates ESBL production and multidrug resistance in pathogenic bacteria.

There is a need to increase awareness on control of infection, as well as ensure proper awareness on antibiotic stewardship and proper personal hygiene to contain the menace of AMR in the studied population.

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