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Prevalence and Antibiogram of Extended Spectrum β- Lactamase Producing *Eschericha coli* and *Klebsiella pneumoniae* Isolated from Urine of Child-Bearing Women

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Abstract:- *Klebisella pneumoniae* and *Escherichia coli* are the major extended spectrum beta-lactamases (ESBLs) producing microorganisms and are responsible for failures in treating urinary tract infections (UTIs) because of its resistance to antibiotics. This study aimed at evaluating the prevalence and antibiogram of ESBLs producing *E. coli* and *Klebsiella* pneumoniae isolated from the urine of child-bearing women. A total of eighty (80) urine samples were collected from both pregnant and non-pregnant women within the age ranges of 15 – 44 years. The samples were cultured on CLED agar for the detection of the organism and identified by other standard microbiological methods. Phenotypic screening test in the presence of ceftazidime (30 *µ*g), cefotaxime (30 *µ*g), and ceftriaxone (30 *µ*g) were used to detect the ESBLs producing organisms. The double disc synergy test (DDT) was used to confirm the ESBL- producing organisms. A disc of amoxicillin + clavulanic acid (20/10*µ*g) was placed at the centre of the Mueller Hilton agar plate and ceftazidime (30 *µ*g), cefotaxime (30 *µ*g), and ceftriaxone (30 *µ*g) were placed at a distance of 20 mm from the Amoxicillin-clavuanic acid disc. Enhanced inhibition zone of any of the discs on the site facing the amoxicillin + clavulanic acid disc was considered as ESBLs producer. The ESBL producers were found more on age range of 26-30 (42.5%). The prevalence rate of ESBL producing organisms were detected in 61.54% (n=70) of pregnant women and 38.46% (n=10) of non-pregnant women (p<0.05). *Escherichia coli* accounted for 28.75% (n=23) of the ESBL producing bacteria while *Klebsiella pneumoniae* accounted for 3.75% (n=3). ESBLs producing *E. coli* and *Klebsiella pneumoniae* showed high resistance to cefotaxime (100%), and ceftriaxone (100%) and completely sensitive to Amikacin (100%) and Iminipen (100%). From this study the antibiotic resistance pattern associated with ESBLs producing *E.coli* and *Klebsiella pneumoniae* is a useful guide for treatment and management of infections caused by ESBLs producing bacteria.

Keywords:- *Extended Spectrum Beta-Lactamases (Esbls). Antibiotic Resistant, Phenotypic Screening, Child-Bearing Age Women, Multidrug Resistance.*

I. INTRODUCTION

Beta-lactamases are enzymes produced by bacteria that break down a particular class of antibiotics called the beta-lactams (Chinyere *et al*., 2020).A**s** the bacteria developed resistance to one type of beta-lactam antibiotic, new antibiotic derivatives (cephalosporins, carbapenems and monobactams), which inturn made the bacteria to continually evolved and developed new set of enzymes described as extended-spectrum beta-lactamases (ESBLs) that can break down the newer derivatives (Chinyere *et al*., 2020). ESBLs are plasmid mediated enzymes that confer resistance to penicillin, third generation cephalosporins and aztreonam but are said to be inhibited by clavulanic acid (Patterson and Bonomo, 2005). The global increase in antibiotic resistance among these uropathogens are due to their ability to hydrolyse virtually all the beta-lactam antibiotics including the third generation cephalosporins and carbpenems enzymes (CTM-M-14 and CTX-M-15) or Ampc β-lactamases and OXA-type beta-lactamases (Laupland *et al*., 2008; Woodford *et al*., 2004). Moreover, antibiotic resistance occurs naturally but misuse of antibiotics in humans and animals have increased the process of resistance (Hackman *et al*., 2021).

ESBL producing bacteria such as *E. coli*, *Klebsiella* sp, *Proteus* sp and *P. aeruginosa* have been found to continuously develop resistance to several antibiotics which has resulted to wide spread cases of chronic urinary tract infections (UTI) in pregnant women (Chinyere *et al*., 2020).The isolation of ESBL positive organisms limit therapeutic options and these patients invariably require urgent antibiotic therapy. It has been widely reported that bacteria habour antibiotic resistance genes which can be horizontally transferred to other bacteria, thus this calls for urgent research on their prevalence and management

(Piddock, 2006).UTI accounts for 10% of all hospital admissions during pregnancy (Lumbiganon *et al*., 2010). Urinary tract infections are the second common complications in pregnant women, which if untreated can adversely affect the health of infant or the pregnant mother (Manjula *et al*., 2013). Pregnant women have four times higher rate of developing UTI compared to non-pregnant women (Rosana, 2016; Vasudevan, 2014). This is as a result of abnormal anatomical and physiological changes that occur during this period (Ferede *et al*., 2012; Vasconcelos-Pereira *et al*., 2013).

Tests for extended spectrum beta- lactamases detection are not routinely done in hospital laboratories despite increase in treatment failures observed for common clinical conditions like urinary tract infection. This ESBL contribute to multidrug resistance among the organisms and detection of these enzymes is crucial to treatment, thus, their detection is therefore significant in preventing treatment failures caused by these pathogens. The aim of the study was to determine the prevalence and antibiogram of extended spectrum beta- lactamase producing *E. coli* and *Klebsiella pneumonia* isolated from the urine of women of child-bearing women.

II. MATERIALS AND METHODS

➢ *Study Population:*

The study engaged some pregnant and non-pregnant mothers attending clinics at some selected Obstetrics and Gynecology (O and G) hospitals within Enugu metropolis. Saint Patrick's hospital, Obiagu and Semino hospital Abakpa, were selected for subject recruitment. Women of childbearing age between the age brackets of 15 to 44 years were recruited for the study. Women that were menstruating and those below the ages of 15 years were excluded for the study.

➢ *Study Design:*

A structured questionnaire was designed to obtain a demographic information from the recruited subjects from both hospitals.

➢ *Ethical Consideration:*

Ethical approval was duly received from both hospitals. Informed consent was sort from each patient or patient relatives before filling the questionnaire and sample collection.

➢ *Sample Size:*

The study was a pilot survey and a total of eighty (80) samples were collected from the recruited patients. The sample size was calculated following the method suggested by Charan and Biswas (2013).

$$\text{Sample size} = \frac{Z_{1-\alpha/2}^2 P(1-P)}{d}$$

- $Z_{1-\alpha/2}^2$ ..... Is standard normal variate (at 5% type 1 error (P<0.005) it is 1.96 and at 1% type 1 error (P<0.01) it is 2.58. As in majority of studies P values are considered significant below 0.05 hence 1.96 is used in formula.
- **p** = Expected proportion in population based on previous studies or pilot studies.
- **d** = Absolute error or precision – Has to be decided by researcher.

➢ *Sample Collection:*

Each patient was given sterile, wide mouthed, and leak proof universal urine container and were asked to collect midstream urine into it. The samples were labeled and sent to the laboratory for microbiological analysis after collection.

➢ *Sample Analysis:*

The urine samples were analyzed microbiologically ensuring aseptic conditions were maintained throughout the analysis to avoid contamination.

➢ *Media Preparation:*

All media employed in the study were weighed and prepared according to the manufacturer's instruction. The media employed included (CLED), MacConkey agar and Mueller Hinton agar.

➢ *Media Inoculation:*

By streak plate technique, the inoculum was aseptically streaked into MacConkey and CLED agar plates and then were incubated at 37^{o}C for 24 hours.

➢ *Identification of Isolates:*

These isolates were presumptively identified based on their colonial characteristics and they were subjected to biochemical tests to further identify them to generic level.

III. ESBL DETECTION METHODS

The isolated *E. coli and Klebsiella pneumoniae* were further screened to determine those that produce Extended Spectrum Beta Lactamases (ESBLs). The first step was to screen for ESBL production by the phenotypic method and then were later confirmed by the phenotypic confirmatory test as outlined by CLSI guidelines (2014).

➢ *Phenotypic Screening for ESBL Production:*

This technique was achieved by the standard disk diffusion method by using ceftazidime (30 μg), cefotaxime (30 μg), and ceftriaxone (30 μg) (Oxoid, UK). The essence of using more than one antibiotic disc in this screening was meant to improve the sensitivity of ESBLs detection, as recommended by CLSI guidelines (2014).

Young culture colonies of *E. coli and Klebsiella pneumoniae* were first suspended into normal saline. The turbidity of the suspension was adjusted at 0.5 McFarland's standard. Thereafter, each of the suspension was inoculated onto Mueller Hinton agar plates with sterile cotton swab. Thereafter, the three antibiotics (ceftazidime (30 μg), cefotaxime (30 μg), and ceftriaxone (30 µg) were placed at agap of 20mm and incubated at 35°C for 18 hours.

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➢ *Phenotypic Confirmation of ESBL Producers:*

This was achieved by using the double-disk synergy (DDS) method on Mueller Hinton agar, as recommended by CLSI guidelines (2014).

Here, a disc of amoxicillin + clavulanic acid (20/10 μg) was placed in the center of the Mueller Hinton Agar plate already inoculated with the isolates, and then cefotaxime (30 μg) and ceftazidime (30 μg) were placed at a distance of 20mm (center to center) from the amoxicillin+ clavulanic acid disc on the same plate. Then each of the plates was incubated at 37ºC for 24 hours.

➢ *Antimicrobial Susceptibility Testing:*

This was done by using Kirby–Bauer disc diffusion technique on Mueller Hinton agar according to the CLSI guidelines (2014).

The following antimicrobial discs were used: amoxicillin/clavulanic acid (20/10 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μ g), ampicillin (10 μg), cephalothin (30 μg), ciprofloxacin (5 μg), nalidixic acid (30 μg), norfloxacin (10 μg), gentamicin (10 μg), amikacin (30 μg), trimethoprim sulfamethoxazole (1.25/23.75 μg), imipenem (30 μg), and chloramphenicol (30 μg) (Oxoid; UK). A typed strain of both *E. coli* and *Klebsiella pneumoniae* were sourced from National Collection of Typed Cultures (NCTC) to serve as an internal control in identification of the isolates, (*E. coli* NCTC 12241 and *Klebsiella pneumoniae* NCTC 13368 respectively).

After overnight incubation of the Mueller–Hinton agar plate with antimicrobial discs at 37˚C, the zone of inhibition was measured in millimeter (mm).

➢ *Statistical Analysis:*

The data were analyzed by statistical package for social sciences (SPSS) and descriptive statistics, chi-square, binary and multivariate logistic regression were employed. P-value<0.05 with 95% confidence interval was considered statistically significant.

IV. RESULTS

➢ *Demographic Characteristics:*

From the questionnaire, it was observed that the highest number of the respondents 40(50%) were secondary school graduates and 28 (35%) respondents that were either B.Sc. or HND holders. However, 10(12.5%) respondents were holders of First Leaving Certificate (FSLC) while 2(2.5%) respondents did not attend formal education(table 1).From the study, the economic status, marital status, different symptoms noticed by the respondents were determined and these results were shown in tables 2, 3 and 4.The difference between those who said "yes" and those who said "no" was highly significant ($P < 0.001$) with respect to the presence of all except one of the symptoms, Dysuria, ($P = 0.823$). While 66.25% of the respondents indicated that they experienced the symptoms during pregnancy, 33.75% said that the symptoms were not experienced during pregnancy ($P < 0.001$). A much higher proportion (81.25%) of the respondents indicated that they had no underlying health problems than those who indicated having underlying health issues ($P < 0.001$) (table 4).

The stage of pregnancy in which those symptoms were noticed as well as frequency of antibiotic intake were determined. These results were shown in tables 5 and 6.The difference in population between those in their first trimester, second trimester and third trimester was highly significant ($p = 0.002$), with third trimesters making up 47.5%, followed by second trimesters (36.25%) and lastly first trimesters (16.25%) (table 5).An overwhelming majority (91.25%) of the participants affirmed that they take antibiotics as many times as they are sick. Thus the difference between the population of those who seldom take antibiotics and those who often take it is statistically significant ($P < 0.001$) (table 6)

Table 1 Educational Qualification

Educational qualification:	**Number**	**Percentage (%)**
(a) FSLC	10	12.5
(b) SSCE	40	50
(c) BSc/HND	28	35
Others please specify	2	2.5
	80	**100**

Table 2 Economic Status of the Respondents

Employment	**Number**	**Percentage (%)**
(a) Civil servant	23	28.75
(b) Entrepreneur	45	56.25
(c) House wife	12	15
	80	**100**

Table 3 Marital Status

Marital status	**Number**	**Percentage (%)**
Married	53	66.2
Not married	27	33.75

Table 4 Determination of the Different Symptoms Noticed by the Respondents

S/N	Item	Yes (%)	No (%)	X^2	P-value	95% CI	
						Lower	Upper
Have you noticed this sort of symptoms							
1	Dysuria	41(51.25)	39(48.75)	0.05	0.823	0.963	1.000
2	Fever	80(100)	0(0.00)	*	0.000	*	*
3	Vomoting	74(92.5)	6(7.50)	57.80	0.000	0.000	0.037
4	Suprapubic pain	67(83.75)	13(16.25)	36.45	0.000	0.000	0.037
5	Nausea	76(95.0)	4(5.00)	64.80	0.000	0.000	0.037
6	Chills	63(78.75)	17(21.25)	26.45	0.000	0.000	0.037
When did you observe any of the symptoms listed above?							
1	During pregnancy	53 (66.25)	27 (33.75)	8.45	0.000	0.000	0.037
Are you having any underlying health problems like diabetes mellitus							
1	Underlying issue	15 (18.75)	65 (81.25)	31.25	0.000	0.000	0.037

Table 5 At What Stage of your Pregnancy did you Notice any of those Symptoms

Responses at 95% CI						
Items	**Yes**	**Percentage (%)**	**X^2**	**P-value**	**Lower**	**Upper**
(a) first trimester	13	16.25	12.025	0.002	0.000	0.037
(b) second trimester	29	36.25				
(c) third trimester	38	47.5				

Difference between variables significant at p-value ≤ 0.05

Table 6 How often do you use antibiotics?

Responses at 95% CI						
Item	**Yes**	**Percentage (%)**	**X^2**	**P-value**	**Lower**	**Upper**
(a) Seldom	7	8.75	54.450	0.000	0.000	0.056
(b) As many times as you are sick	73	91.5				
(c) Not at all	0	0.0				
	80	**100**				

➢ *Determination of ESBL Producers from the Pregnant Women According to Age:*

ESBL producers from pregnant women according to age were determined. It was found out that the highest ESBL producers were found from women of age 26-30, which had 42.31% (table7).

Table 7 ESBL Producers from the Pregnant Women According to age

Age	Total isolates (%)	ESBL positive (%)	ESBL negative (%)
15-20	10(12.5)	4 (15.38)	6 (11.11)
21-25	19 (23.75)	7 (26.92)	12 (22.22)
26-30	34 (42.5)	11 (42.31)	23 (42.59)
31-35	11 (13.75)	3 (11.54)	8 (14.8)
36-40	4 (5.0)	1 (3.85)	3 (5.56)
41-44	2 (2.5)	0 (0.00)	2 (3.7)
Total	80 (100)	26 (100)	54 (100)

➢ *Occurrence of the two Isolates Escherichia Coli and Klebsiella Pneumoniae in Urine:*

Among the 80 urine samples collected, 35(43.77%) were *E. coli* and 20(25%) emerged *Klebsiella pneumoniae* while 25(31.25%) were other organisms. Phenotypic screening revealed that the percentage of *E. coli* and *Klebsiella. pneumoniae* that produced Extended Spectrum Beta-Lactamases (ESBLs) were 28.75% and 3.75% respectively (table 8).

Table 8 Occurrence of two the Isolates (*E. Coli* And *Klebsiella Pneumoniae*) in Urine

Organism	Number of Isolates (%)	ESBLs positive (%)	ESBLs Negative (%)	X^2	P-value	95% CI	
						lower	upper
E.coli	35(43.77)	23(28.75)	12(15)	3.45	0.063	0.026	0.149
Kleb.pneumoniae	20(25)	3(3.75)	17(21.25)	9.80	0.002	0.000	0.037
Other species	25(31.25)	NA	NA				
Total	**80**	**26**	**29**				

➢ *Phenotypic Screening of ESBLs Producing Isolates:*

A higher number of *E. coli* was resistant against all five antibiotics tested than was susceptible. However, there was no significant difference (P > 0.05) between the resistant and the susceptible organisms except in the test with the antibiotic, Cefotazidime (P = 0.011). *Klebsiella pneumoniae* was 100% resistant to 2 antibiotics (Gentamicin and Penicillin), while 95% of *Klebsiella pneumoniae* was also resistant to Cefotaxime, Cefotazidime and ceftriaxone (table 9).

Table 9 Phenotypic Screening of ESBLs Producing Isolates

S/N	Antibiotics	E.coli (n=80)		X^2	P-value	95% CI		Klebsiella spp. (n=20)		X^2	P-value	95% CI	
		R (%)	S (%)			L	U	R (%)	S (%)			L	U
1	Cefotaxime	23(28.75)	12(15)	3.457	0.063	0.009	0.220	19(95)	1(1.25)	16.200	0.000	0.000	0.082
2	Cefotazidime	25(31.25)	10(12.5)	6.429	0.011	0.000	0.082	19(95)	1(1.25)	16.200	0.000	0.000	0.082
3	Gentamicin	20(25)	15(18.75)	0.714	0.398	0.349	0.680	20(100)	0	*	*	*	*
4	Penicillin	19(23.75)	16(20)	0.257	0.612	0.667	0.933	20(100)	0	*	*	*	*
5	Ceftriaxone	22(27.5)	13(16.25)	2.314	0.128	0.000	0.178	19(95)	1(1.25)	16.200	0.000	0.000	0.082

* Difference between variables are statistically significant at P-value ≤ 0.05

- **Key:**

✓ R = Resistance
✓ S = Susceptible
✓ = No values because all test subjects were resistant.

➢ *Comparing zones of inhibition (mm) of E. coli isolates and E. coli (NCTC12241) against antibiotics:*

There was no significant difference between zones of inhibition of ***E. coli*** Isolates and ***E. coli* (NCTC12241)** against each of the six antibiotics used (table 10). Interestly, there was no zone of inhibition of *Kleb. pneumoniae* against Cefotaxime antibiotic, while there were 3 zones of inhibition of *Klebsiella pneumoniae* strains against that antibiotics (table 11).

Table 10 Comparing Zones of Inhibition (mm) of *E. coli* Isolates and *E. coli* (NCTC12241) against Antibiotics

S/N	Item	ZoI (NCTC 12241)	ZoI(Isolates)	X^2	P-value	95% CI	
						Lower	Upper
1	Gentamicin (CN)	15	11	0.615	0.433	0.559	0.766
2	Ceftazidime (CAZ)	20	25	0.556	0.456	0.390	0.610
3	Penicillin G (P)	8	13	1.190	0.275	0.293	0.507
4	Amoxil + Clavulanic acid	15	16	0.032	0.857	0.963	1.000
5	Cefotaxime (CTX)	14	10	0.667	0.414	0.467	0.683
6	Ciprofloxacin (CIP)	5	8	0.692	0.405	0.454	0.671

Table 11 Comparing Zones of inhibition (mm) of *Klebsiella* Isolates and *Klebsiella pneumonia* (NCTC 13368) against antibiotics.

S/N	Item	ZoI (NCTC 12241)	ZoI(Isolates)	X^2	P-value	95% CI	
						Lower	Upper
1	Gentamicin (CN)	16	10	1.385	0.239	0.281	0.494
2	Ceftazidime (CAZ)	13	15	0.143	0.705	0.818	0.957
3	Penicillin G (P)	20	12	2.000	0.157	0.123	0.302
4	Amoxil + Clavulanic acid	15	7	2.909	0.088	0.072	0.228
5	Cefotaxime (CTX)	0	3	*	*	*	*
6	Ciprofloxacin (CIP)	22	20	0.095	0.758	0.757	0.918

➢ *Susceptibility Profile of ESBLs Producing E. coli and Klebsiella pneumoniae:*

In the tests with *E. coli*, in two separate tests with two different antibiotics (Cefotaxime and Cefriaxone, representing 14% of the antibiotics tested), all (100%) of the test *E. coli* were resistant. In addition, more than half (57%) of the test *E. coli* was resistant to 86% (12 antibiotics) of the antibiotics. Again, in two separate tests with two different antibiotics (Amikacin and

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Iminipem, representing 14% of the antibiotics tested), all (100%) of the test *E. coli* were susceptible. Thus between 57% and 100% of the test *E. coli* were resistant to 12 (86%) different antibiotics, with the difference between the number of resistant and susceptible test subjects being significant ($P < 0.05$) in all except one of the 12 tests, the test with the antibiotic Norfloxacin.

With respect to tests conducted using *Klebsiella pneumoniae*, in four separate tests (using the antibiotics Cefotaxime, Cefriaxone, gentamicin and AMC), all 3 (100%) test subjects were resistant. Furthermore, between 1 and 2 test subjects were resistant to different antibiotics used in 8 separate tests. All 3 (100%) test subjects were susceptible to two different antibiotics (Amikacin and Iminipem).

It is interesting to note that 100% of both *E. coli* and *Klebsiella pneumoniae* test subjects were susceptible to both Amikacin and Iminipem antibiotics.

Table 12 Susceptibility Profile of ESBLs Producing E. *coli* and *Klebsiella pneumoniae*

	ESBL-positive *E. coli*				**95% CI**		**ESBL positive *Klebsiella pneumoniae***				**95% CI**	
Antibiotics	**R**	**S**	X^2	**P-value**	**Lower**	**Upper**	**R**	**S**	X^2	**P-value**	**Lower**	**Upper**
Cefotaxime	23	0	*	*	*	*	3	0				
Cefriaxone	23	0	*	*	*	*	3	0				
Ceftazidime	20	3	12.565	0.000	0.000	0.122	2	1	0.333	0.564	0.878	1.000
AMC	18	5	7.348	0.007	0.000	0.122	3	0				
Cephalothin	22	1	19.174	0.000	0.000	0.122	2	1	0.333	0.564	0.878	1.000
Ampicillin	21	2	15.696	0.000	0.000	0.122	2	1	0.333	0.564	0.878	1.000
Gentamicin	20	3	12.565	0.000	0.000	0.122	3	0				
NA	17	6	5.261	0.022	0.000	0.127	1	2	0.333	0.564	0.878	1.000
Amikacin	0	23	*	*	*	*	0	3				
CIP	20	3	12.565	0.000	0.000	0.122	2	1	0.333	0.564	0.878	1.000
C	19	4	9.783	0.002	0.000	0.122	2	1	0.333	0.564	0.878	1.000
Norfloxacin	13	10	0.391	0.532	0.614	0.951	1	2	0.333	0.564	0.878	1.000
Iminipem	0	23	*	*	*	*	0	3				
SXT	18	5	7.348	0.007	0.000	0.122	2	1	0.333	0.564	0.878	1.000
TOTAL	234	88	66.199	0.000	0.000	0.122	26	16	2.381	0.123	0.000	0.202

Difference between resistant and susceptible pathogens statistically significant at P-value < 0.05

- Key
- ✓ R = Resistance
- ✓ S = Susceptible
- ✓ = No values because all test subjects were either resistant or susceptible
- ✓ AMC=amoxicillin-clavulanic acid, NA= nalidixic acid, CIP=ciprofloxacin, SXT=trimethoprim-sulfamethoxazole, C=chloramphenicol

V. DISCUSSION

The emergence of ESBL producing bacteria in pregnant women has become a public health issue thereby resulting in drug resistance and treatment failure. In this study, some factors that could be responsible for urinary tract infection during pregnancy were looked into, such as: educational qualification, economic background, marital status, maternal age and presence of underlying health challenges. On the educational qualification of the women recruited from this study, it was found out that, out of the eighty (80) women, 50% of them had SSCE while 12.5% had FSLC and 35% had BSC/HND (table 1). The reason why the UTL is high among them could be due to the fact that most of them are illiterates and could not define when they are sick or not. This is in line with the work of Rekha *et al*. (2015) who remarked that the high prevalence of UTI in illiterate women might be due to poor genital hygienic practices. Considering the economic status of the women recruited for the study, it was found that most of the women were entrepreneurs representing 56.2% of the total respondents while 28.8% were government employed and 15% were not employed (Table 2). This could be as a result of availability of fund to assess hospital services during pregnancy, because majority believed that it is more expensive to visit antenatal clinics than to seek for care from elderly mothers and local delivery centers. Similarly, majority of the respondent are legally married representing 66.3% of the total respondents while 33.7% were either single parent or got pregnant out of wedlock (Table 3). This could also be due to lack of knowledge of importance of antenatal visits or may be lack of fund to seek for proper clinical attention by a qualified midwife. A total of 53 (66.25%) respondents acknowledged that it was during their pregnancy that they noticed symptoms such as dysuria, fever, vomiting, suprapubic pain, nausea and chills while 47(58.75%) said they did not notice these symptoms during pregnancy (table 4).This could be as a result of lack of

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health awareness and ability of the women to differentiate a symptom from another. The difference in population between those in their first trimester, second trimester and third trimester was highly significant (p=0.002), with third trimesters making up 47.5%, followed by second trimesters (36.25%) and lastly first trimesters (16.25%) (Table 5). These results in this work is higher than the result gotten from the work of Chinyere *et al*. (2020), who had 10% in first trimester, 19% in second trimester a(nd 8% in third trimester. This study is in agreement with the work of Ranjan *et al*.(2017) whose highest incidence of UTI is seen in third trimester (48%) followed by second trimester (45%). This could be due to subject's exposure level and personal hygiene (Chinyere *et al*., 2020). The result of this study was highly significant at (p=0.002) which means that there is association between gravidity and incidence of UTI in pregnancy.

High percentage of the respondents (91.25%) said they use antibiotics as many times as they are sick, while 7(8.75%) said they use it seldom (table 6). This indiscriminate use of antibiotics as observed in this present study by most of the women may be suggestive of the high occurrence of this ESBL among these women and also pose a risk for the development of UTI. This study is in agreement with the work of Colodner *et al.* (2004) who suggest that the greater exposure to antibiotics may lead to development of selection pressures. Highest ESBLs producing isolates were witnessed among the age group range of 26-30, a total of 42.31% (n=11) isolates came from these age range. They were followed by those within the age range of 21-25 which had ESBLs production at 26.92% (n=7). A total of 15.38% ESBLs producing isolates were recovered among those with the age range of 15-20. However, 3.85% (n=1) respondents within the age range of 36-40 had the least prevalence of ESBLs producers. Those within the age range of 41-44 had no ESBLs producers (table 7). This is in line with the work done by Vachvanichsanong *et al*. (2021), who had lower prevalence of ESBL producers within the same age group. They noted that age has no association with ESBL.

The prevalence and ESBL- producing rates of *Escherichia coli* and *Klebsiella pneumoniae* in urine of pregnant women in this study was 43.77% and 28.75%, 25% and 3.75% respectively (Table 8). These results are close to the one obtained from the works of Chinyere *et al*. (2020) who got 37% as prevalence of *Escherichia coli* and ESBL-producers in *Klebsiella pneumoniae* as 19%; Ranjan *et al*. (2017) who had 35% as prevalence rate of UTI; Viet *et al*., 2021 who obtained prevalence rate of *Escherichia coli* as 21% and ESBL as 11%, prevalence of *Klebsiella pneumoniae* as 8% and ESBL- producers in *Klebsiella pneumoniae* as 3%.This result is close to the work of Melaku (2020), who obtained 15.8% as prevalence of ESBL. This study was also consistent with the works of Aboderin *et al.*, 2009 and Oluremi *et al*. 2011 who reported that *E coli* and *Klebsiella* sp. are most prevalence uropathogens. Prevalence rate of *Klebsiella pneumoniae* 12.6%, 20% and 21.6% were obtained by Dong *et al*. (2010), Onwuezobe and Orok, (2015) and Motayo *et al*. (2013) respectively. The reason for high rate of *Escherichia coli* was due to the significant abundance of *E coli* as fecal flora which in turn through contamination ascend through genitalia to cause UTI and due to numerous virulence factors used for colonization and invasion of the urinary epithelium such as p- fimbriae or pili adherence factors which mediate the attachment of *E coli* to uroepithelial cells (Melaku, 2020). The rise in the prevalence of ESBL-producing uropathogens might be attributed to the habit of empirical treatment practice without drug susceptibility testing, poor compliance of patients with prescribed drugs, difference in the study population and health-care trends.

Phenotypic screening of the ESBLs producing *E. coli* and *Klebsiella pneumoniae* isolates indicated *E. coli* was resistant against all five antibiotics tested. However, there was no significant difference ($P > 0.05$) between the resistant and the susceptible organisms except in the test with the antibiotic, Cefotazidime ($P = 0.011$). *Klebsiella pneumoniae* was 100% resistant to 2 antibiotics (Gentamicin and Penicillin), while 95% of *Klebsiella pneumoniae* was also resistant to Cefotaxime, Cefotazidime and ceftriaxone (Table 9). In the present study, it was observed that *E. coli* and *Klebsiella pneumoniae* isolates were susceptible to amikacin and imipenem 100% respectively (Table 12). This is in agreement with the works of Melaku, (2020) and Siraj *et al*., (2015) who had the same result. The possible reason for this rise in multidrug resistance might be repeated, inappropriate and incorrect use of antimicrobial agents in empirical treatment and poor infection control strategies which in turn raise the prevalence of resistant microorganisms. These findings imply that the use of these antibiotics for the treatment of urinary tract infections caused by ESBL-producing strains may not be effective and may result in a significant amount of treatment failure. On the other hand, ESBL positive strains can respond better for carbapenem drugs such as imipenem which could be a better treatment option.

VI. CONCLUSION

It was observed from this study that pregnant women had high prevalence of UTI and ESBL- producing *E. coli* and *Klebsiella pneumoniae* which may transmit to neonates, thereby resulting to early onset of neonatal sepsis. Moreover, a significant increase of resistance to the antibiotics used in this study by the two bacteria were alarming, hence, there is need for instant UTI culture screening of pregnant women especially those with previous history of UTI. It is therefore important to enlighten them on the appropriate prescription and use of antibiotics.

- *Conflict of interest statement*

Authors declare that they have no conflict of interest.

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