



## Bacterial resistance and virulence markers in urine of HIV patients and pregnant women in Port Harcourt, Nigeria

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**Abstract:** The present study was carried out to ascertain resistance pattern in *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in urine samples of HIV patients and pregnant women attending the University of Port Harcourt Teaching Hospital. A total of one hundred and thirty (130) urine samples were collected from HIV patients and equal number for pregnant women. The midstream urine samples were cultured on cystine lactose electrolyte deficient agar (CLED) media. Isolates were identified for the presence of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Antibiotic susceptibility testing was performed on identified isolates by Kirby-Bauer's disc diffusion method. Results from the study revealed that 2.31 % men and 10.76% female were positive for HIV while 29.23% were positive for pregnant women. Four uropathogens occurred in HIV patients while eight pathogens in pregnant women with *Escherichia coli* (72.22%) and *Klebsiella pneumoniae* (34.04) as the highest occurring uropathogens respectively. The least occurred uropathogens were *Pseudomonas aeruginosa* and *Proteus* sp. (5.56% for each) in HIV patients and *Enterobacter aerogenes* (2.13%) and *Alcaligenes faecalis* (2.13%) in pregnant women. The rate of infections were highest among pregnant women in their third trimesters (65.75%) and HIV patients less than 5 years on antiretroviral drugs (52.94%). All isolates were highly resistant to Imipenem/Cilastatin, Amoxicillin and Cefuroxime while most isolates were 100% susceptible to Ofloxacin and Levofloxacin antibiotics. *Pseudomonas aeruginosa* showed high multidrug resistance in pregnant women. There is need to create awareness on uropathogens, factors leading to their acquisition, prevention, testing and treatment should be increased at trimesters in pregnancy and HIV patient's routine check-up

**Keywords:** Antibiotics, HIV patients, Pregnant women, Resistance, Uropathogens

**Introduction:** Even though there is progress in the understanding of the pathogenicity and treatment of urinary tract pathogens, they still remained a major problem in the clinical field. Urinary tract infection (UTI) is the presence of microorganisms in specific amount that are pathogenic when seen in the intestinal tract of humans, they are called uropathogens. They are community acquired pathogens which poses serious public health issues, thereby decreasing the quality-of-life (Olowe et al., 2015). Uropathogens resistance in urine is encountered frequently people with compromised immune-system especially Human immune-deficiency virus (HIV) patients and pregnant women (Nelson & Good, 2015 & Nicolle, 2008). In recent times several measures have been put in place to deal with this problem. These measures have reduced the rate of morbidity and mortality leading to management of these uropathogens. However, recent evidences in researches carried out by workers showed that the success achieved through antibiotic are threatened by antimicrobial resistance especially bacterial resistance in both hospital and

community settings. Globally, resistance of antibiotics by microorganisms has become a serious concern which is currently estimated to account for more than 700,000 deaths per year worldwide and high medical cost, treatment, morbidity and mortality (Oneil, 2016). Use of antibiotics without prescription, miss-use and over-use are major factors that has escalated antimicrobial resistance. HIV patients and pregnant women are more at risk due to compromised immunity in HIV and hormonal and physiological changes during pregnancy

There is an accelerating rate of antibiotic resistance in developed and nations with industrialization where people self-medicate antibiotics without appropriate laboratory test. This in turn has led to HIV patients and pregnant women to acquire high level of antibiotics resistance. Illnesses caused by antibiotic resistance exhibited by these uropathogens can lead to complications, prolonged hospitalization and high cost of treatment. The need to understand and eliminate these uropathogens among these targeted individuals will go a long way in reducing morbidity and mortality and also increases healthy life span of these two segments of people. This study was thus carried out to determine bacterial resistance and virulence markers in *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in urine samples of HIV patients and pregnant women attending university of Port Harcourt Teaching Hospital, Rivers State.

## **Materials and methods:**

### **Study Area**

The study area for this study is Port Harcourt, the capital of Rivers State, Nigeria. Port Harcourt is known as the fifth largest city in Nigeria after cities like Kano, Ibadan, Lagos and Benin. Port Harcourt is located at Niger-Delta, and often known for its rich culture and tourism. One of the largest hospitals is located in Port Harcourt, and it serves as a referral hospital.

### **Study Design**

The study was designed to investigate and compare the prevalence of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and their antibiotic susceptibility pattern among HIV patients and pregnant women attending university of Port Harcourt, Teaching Hospital. Rivers State, Nigeria.

### **Ethical Considerations**

Ethical considerations and approval for the study was gotten from the Research and Ethics Committee of University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Rivers State, Nigeria in accordance with the ethics for research involving human subjects.

### **Sample Size Determination for HIV Patients and Pregnant Women with Urinary Tract Infections**

The sample size for study was determined using the methods of Charan and Biswas, (2013) following the formula for comparison of two proportion.

$$n = \frac{(Z^* + Z^{pwr})^2 \{P_1(100\% - P_1) + P_2(100\% - P_2)\}}{(P_1 - P_2)^2}$$

n = 129 antenatal patient and 129 HIV patients attending anti-retroviral clinic from 1 year and above.

### **Study Population**

The study population consist of HIV positive patients who have been attending the antiretroviral clinic for minimum of one year and above at the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Rivers State, Nigeria, pregnant women attending the antenatal clinic at the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Rivers State, Nigeria and ten (10) healthy individuals (five males and five females) who were neither HIV positive nor pregnant. These individuals served as control with 2 individuals (male and female) for age intervals; 11-20, 21-30, 31-40, 41-50 and 51-60. The comparison in this work was between HIV positive individuals and pregnant women.

### **Sample Collection**

A total of 130 mid-stream urine specimen was collected from HIV patients and equal number for pregnant women attending anti-retroviral clinic and ant- natal clinic respectively at UPTH. The containers were tightly closed and labelled with serial numbers, date of collection and codes for individual patient, age, years on anti-retroviral drugs and trimesters in pregnancy. The urine specimen was processed within an hour after collection in Medical Microbiology laboratory, University of Port Harcourt, Rivers State.

### **Urine Specimen culture**

A 0.05mm sterile wire loop of was dipped into the urine specimen and was smeared aseptically and uniformly on a sterile Cysteine Lactose Electrolyte Deficient Agar (CLED) plate by the streak method. The plates were incubated for 24-hour at 37°C. Cultures that had growth up to 200 colonies equivalent to 10<sup>5</sup>cfu/ml of urine was recorded as being significant at the other hand, cultures having less than 200 colonies were seen as non-significant.

### **Identification of Isolates**

The morphological characteristics of the isolates were recorded, they were sub cultured to get pure colonies and were stored on agar slants at 4<sup>0</sup>C for other test for confirmation using standard microbiological techniques. Gram staining and other test such as motility test and biochemical characterization like catalase test, coagulase test, oxidase test, indole, citrate utilization test, urease test, Voges – Proskauer test and sugar fermentation test such as glucose, lactose, mannose, galactose, fructose and maltose fermentation were further carried out to identify these organisms.

### **Antibiotic susceptibility Test**

The identified uropathogens were subjected to antibiotics sensitivity testing using disc diffusion techniques. Gram negative multidisc (Celtech diagnostic: Belgium inc ) consisting of antibiotics (such as: Amoxicillin clavulanate (AUG)-30ug, Cefotaxime (CTX)-25ug, Imipenem/cilastacin (IMP)-10/10ug, Ofloxacin (OFX)-5ug, Gentamycin (GN)-10ug, Nalidixic acid (NA)-30ug, Nitrofurantoin (NF)-300ug, Cefuroxime (CXM)-30ug, Ceftriaxone sulbactam (CRO)-45ug, Ampiclox (ACX)-10ug, Cefexime (ZEM)-5ug and Levofloxacin (LBC)-5ug were tested and interpreted in accordance with zone size interpretative chart using the Kirby Bauer method.

## Results

Table 1 shows the total participant involved in the study, while sex distribution of positive and non-positive cases among males and females study population are shown in table 2. Findings from the study showed that HIV patients had 17(13.01%) positive cases, while pregnant women had 38(29.23%) positive cases for uropathogens. Percentage infection rate according to age revealed that ages 41-50 and 31-40 for HIV patients and pregnant women respectively were the most infected. Females less than 3 years at the anti-retroviral clinic were significantly infected than the males while females on 3<sup>rd</sup> trimester had more of these organisms occurring in pregnant women (Table 3).

The prevalence and distribution of uropathogens in HIV patients showed that *Escherichia coli* (72.22%), *Pseudomonas aeruginosa* and *Proteus* sp. (5.56%). The prevalence and frequency of uropathogens in pregnant women was lower with *Klebsiella pneumoniae* (34.04%), *Alcaligenes faecalis* (2,13%) and *Enterobacter aerogenes* (2.13%) as presented in Table 5. Percentage distribution of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* identified in HIV patients showed *Escherichia coli* 76% as the highest occurring uropathogens among the 3 organism and pregnant women showed 42% for *Klebsiella pneumoniae* as the most occurred as shown in figure 1 and 2.

*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* had the highest occurrence in HIV patients less than 5 years on antiretroviral drugs while they occurred most in pregnant women in their third trimester in pregnancy (figure 3). The percentage resistance of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* from HIV patients and pregnant women to antibiotics tested revealed that all isolates were highly resistant to Amoxicillin and imipenem and highly susceptible to Ofloxacin and Levofloxacin (Figure 4 and 5). In HIV patients *Escherichia coli* exhibited 100% resistance to Amoxicillin and Imipenem and also sensitive to Ofloxacin, Gentamicin, Nitrofurantoin Ceftriaxone and levofloxacin. *Klebsiella pneumoniae* was 100% resistant to all antibiotics except Levofloxacin, Nitrofurantoin and ofloxacin while *Pseudomonas aeruginosa* was resistant to all antibiotics except for Levofloxacin and Ofloxacin.

In pregnant women, *Escherichia coli* showed 100% resistance to Amoxicillin, Ampiclox and 90% resistance to Ofloxacin and Levofloxacin. *Pseudomonas aeruginosa* exhibited 100% resistance to all antibiotics tested except for Gentamicin, Nitrofurantoin, Levofloxacin and ofloxacin. *Klebsiella pneumoniae* was highly resistant to all antibiotics except for Nalidixic acid, Gentamicin, Ofloxacin and Levofloxacin.

**Table 1: Total participants**

sex	HIV (n-130) (%)	Pregnant women (n-130) (%)	Control (n-10)
Male	32(24.1)	0(0)	5
female	98(75.4)	130(100)	5

**Table 2: Sex distribution of positive and non-positive cases among males and females study population**

Sex (males and females affected)	Positive Growth in HIV patients (n-130) (%)	Non-positive (n-130)	Total
male	3(9.38)	29((90.62)	32(24.62)
female	14(14.29)	84(85.71)	98(75.38)
Total	Growth in pregnant women (n-130) (%)		130(100)
Male	N/A	N/A	N/A
female	38(29.23)	92(70.77)	130(100.00)
control	0(0)	0(0)	0(0)
Gross total (n 260)	53(20.38)	207(79.62)	260(100.00)

**Table 3 Percentage infection rate according to age**

Age	HIV patients (n-17) (%)	Pregnant women (n-38) (%)
1--10	0(0)	0(0)
11--20	0(0)	0(0)
21--30	2(11.76)	15(39.47)
31--40	2(11.76)	21(55.27)
41--50	8(47.06)	2(5.26)
51--60	4(23.53)	0(0)
61--70	1(5.88)	0(0)

**Table 4: Percentage infection rate according to years on anti-retroviral drugs and trimesters in pregnancy**

years on anti-retroviral drugs	Infection rate (n-17) (%)
1--5	9(52.94)
6--10	5(29.42)

11--15	1(5.88)
16--20	2(11.76)
<b>Trimesters in pregnancy</b>	<b>Infection rate (n-38)</b>
1st	3(7.89)
2nd	10(26.32)
3rd	25(65.79)

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**Table 5: Prevalence and Distribution of Uropathogens in HIV Patients and Pregnant Women**

<b>Uropathogens in urine of HIV patients</b>	<b>prevalence</b>	<b>percentage</b>
<i>Escherichia coli</i>	13	72.22
<i>Klebsiella pneumoniae</i>	3	16.66
<i>Pseudomonas aeruginosa</i>	1	5.56
<i>Proteus sp.</i>	1	5.56
<b>Total</b>	<b>18</b>	<b>100</b>
Uropathogens in Urine of pregnant women		
<i>Escherichia coli</i>	12	25.53
<i>Klebsiella pneumonia</i>	16	34.04
<i>Pseudomonas aeruginosa</i>	10	21.27
<i>Proteus sp.</i>	3	6.38
<i>Alcaligenes faecalis</i>	1	2.13
<i>Enterobacter aerogenes</i>	1	2.13
<i>Staphylococcus aureus</i>	2	4.26
<i>Corynebacterium sp.</i>	2	4.26
<b>Total</b>	<b>47</b>	<b>100</b>

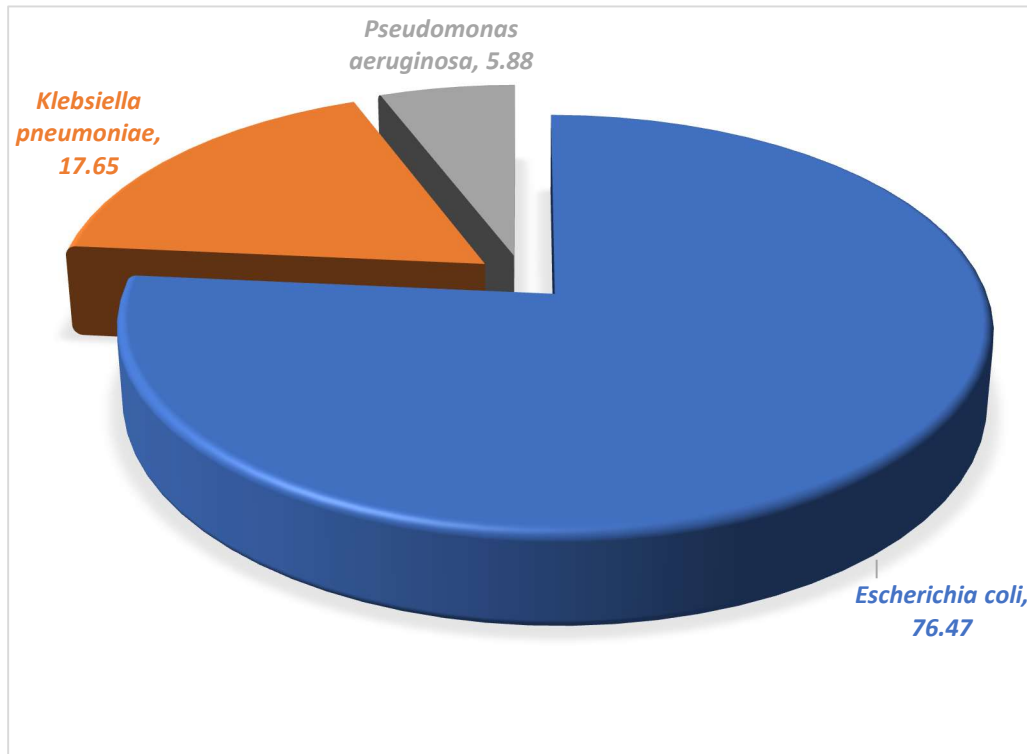


Figure 1: Percentage distribution of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* identified in HIV patients attending anti-retroviral clinic

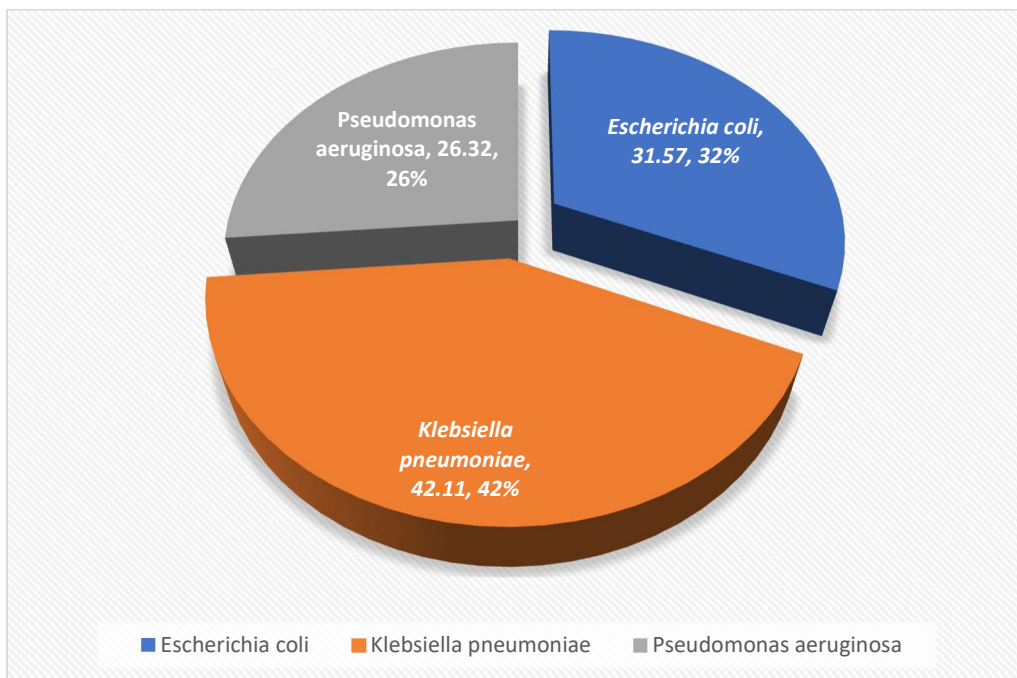
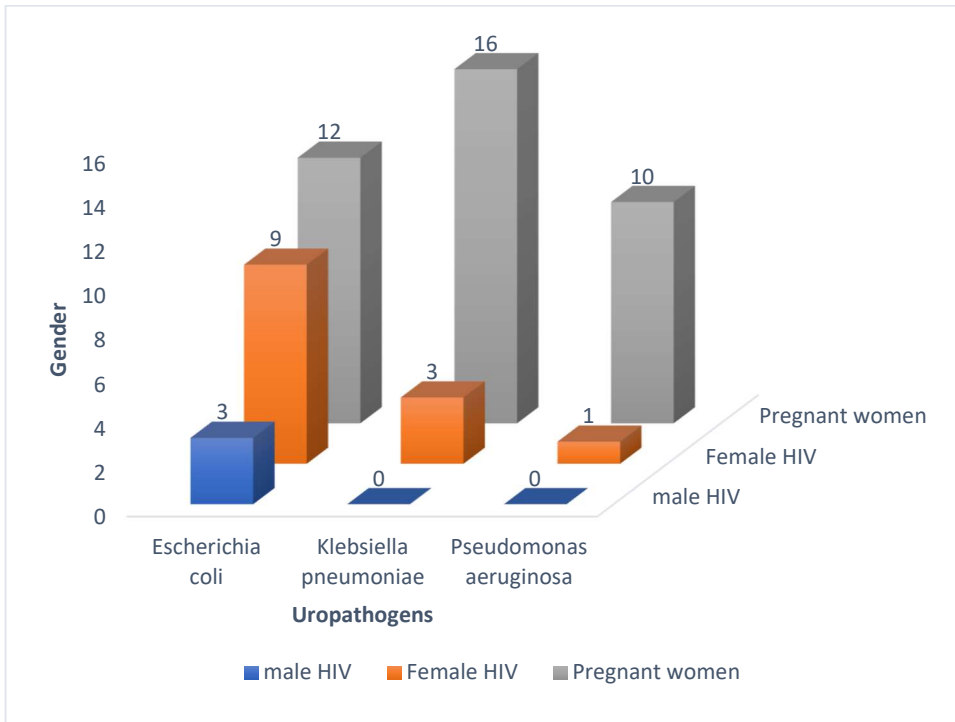
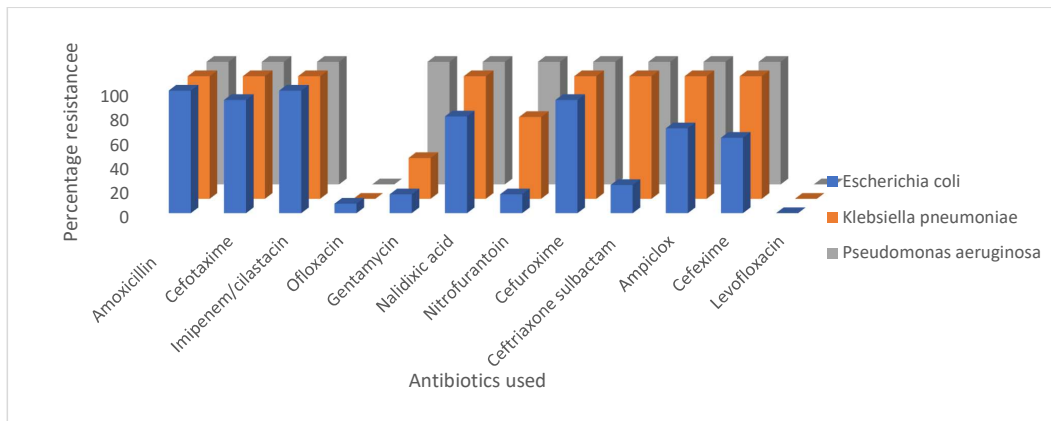


Figure 2: Percentage distribution of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* identified in pregnant women attending ante-natal clinic



**Figure3: Prevalence of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa***

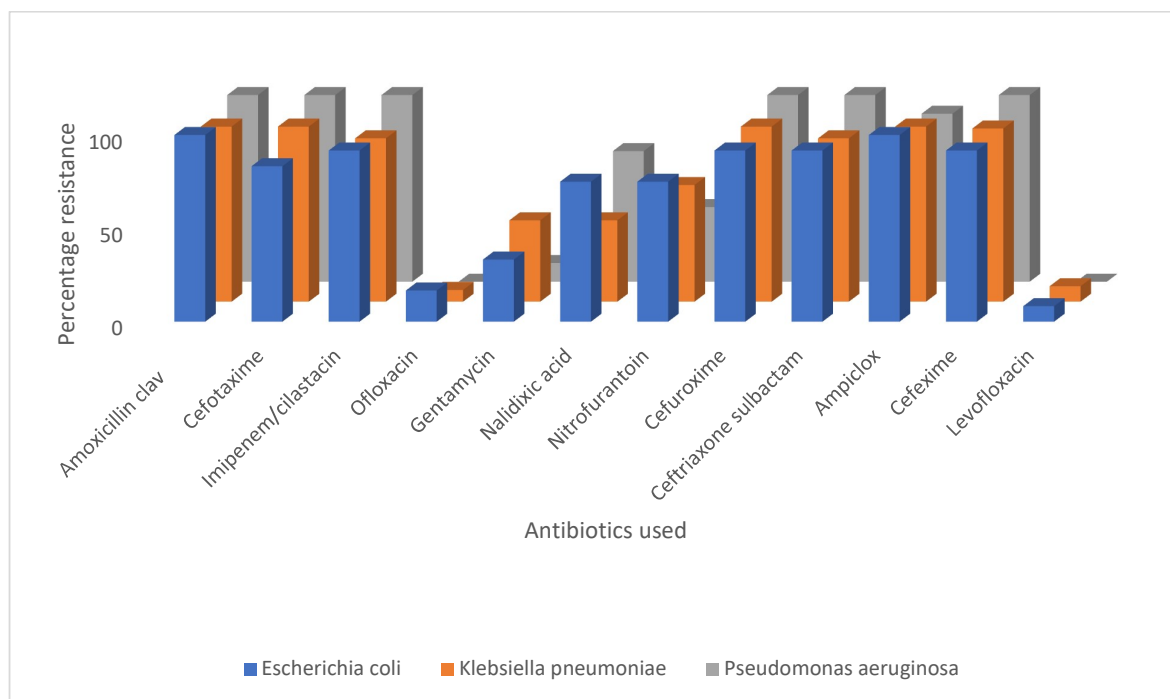
**In HIV patients and pregnant women in respect to gender**



**Figure 4: Percentage resistance of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas***

***aeruginosa* from HIV patients to antibiotics tested**





**Figure 5: Percentage resistance of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* from pregnant women to antibiotics tested**

## Discussion

The present study was carried out to detect the antibiotic susceptibility patterns and virulence makers in *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in urine of HIV patients and pregnant women. The overall prevalence of uropathogens among HIV patients in the present study was 13.09%. This is in-line with other findings carried out by Debaïke et al. (2014) and Tessema et al. (2020) with prevalence of 12% and 10.3% respectively. Other findings showed both low 5.8% and high 21.1% prevalence (Sheyin et. al., 2018; Olowo et al., 2015). This could be due to the size of sample, the level of the immune status of participants in the study and variations geographically. It was observed that pregnant women had a prevalence of 29.23%. This correlates with 31.6% reported by Jido et al (2006). Some investigators reported a high prevalence of 56% prevalence (Gayathri et. al. 2018). In-addition, female HIV patients had a higher prevalence of 10.78% compare to male HIV patients (2.29%). This shows that females (Female HIV patients and pregnant women) are more predisposed to uropathogens more than the males due to the anatomical differences in males and females. The micro-flora alteration of the vagina can lead to uropathogen colonization thus increasing the

high risk of females to urinary tract infection. A drier environment at the urethra and anus distance to the urethra meatus seen in males is responsible for reduced uropathogens. In-contrast, the findings of the current study is not comparable to a study conducted in Calabar that reported a high prevalence of uropathogens in males than females (Inyang-Etoh et. al. 2009)

A high infections rate was recorded in participants between the ages of 41-50 in HIV patients and 31-40 in pregnant women. The high rate in HIV patients could be as a result of high sexual activities associated with this group and some of them in hiding their HIV status tends to hide infections and treat secretly. For pregnant women between 31-40, this is the age at which majority of pregnancies occurs. Pregnant women in their third trimesters of pregnancy show a high infection rate. This is because infections with uropathogens increases with pregnancy. The reason for this is due to increased bladder volume, expansion and expanded ureter. In this study, the uropathogens were more of gram-negative organisms in both groups. This in-line with the findings of Alshabi et al. (2019). The most predominant uropathogen in HIV patients was *Escherichia coli* (72-22%) while in pregnant women, *Klebsiella pneumoniae* (25.53%). This agreed with the finding of Serkadis, et. al. (2014). Alshabi et al. (2019) who reported 73.68% for *Escherichia coli* and Agbagwa & Ifeanacho (2015). In contrast, this study was inconsistent with findings reported at Ebonyi state, Nigeria in which predominant isolates were *Staphylococcus aureus* (Ifeanyi, et. al.2013), Tamil Nadu, India were *Pseudomonas aeruginosa* was predominant (Xavier et al., 2015). In Cape Coast and Ghana *S. aureus* was observed to be most the predominant (Skrat-Klapaczynska, et. al., 2018). The difference in the type of bacterial isolates maybe due to sample collection techniques, personal and environmental hygiene.

All isolates were susceptible to Ofloxacin and Levofloxacin this is consistent with findings of Onoh, et. al. 2013 who reported that *Escherichia coli* and *Klebsiella pneumoniae* were sensitivity to the two antibiotics while most isolates showed resistance to other antibiotics. In pregnant women, *E coli* showed 100% resistance to Amoxicillin, Ampiclox and up to 90% resistance to Nitrofurantoin and other antibiotics tested. This is in line with the findings of Alshabi et al. (2019) who reported that *Escherichia coli* from pregnant women were resistant to Amoxicillin and Nitrofurantoin. *Pseudomonas aeruginosa* exhibited 100% resistance ta all antibiotics used except for Gentamicin, Nitrofurantoin, Levofloxacin and ofloxacin. These two organisms exhibited high level of multidrug resistance since they were resistant to more than 3 classes of antibiotics. *Klebsiella pneumoniae* were highly resistant to all antibiotics except for Nalidixic acid, Gentamicin, Ofloxacin and Levofloxacin. In HIV patients. *Escherichia coli* show 100% resistance to Amoxicillin and imipenem but highly sensitive to most antibiotics which included Ofloxacin, Gentamicin, Nitrofurantoin Ceftriaxone and levofloxacin. *Klebsiella pneumoniae* was 100% resistant to all antibiotics except Levofloxacin, Nitrofurantoin and ofloxacin while *Pseudomonas aeruginosa* was resistant to all antibiotics except for Levofloxacin and Ofloxacin. This result showed that *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* exhibited high level of multidrug resistance

## Conclusion

The study showed that uropathogens were present in urine of HIV patients and pregnant women. It also showed that Levofloxacin and Ofloxacin were the best antibiotics for the treatment of these uropathogens among this targeted group. Antimicrobial susceptibility testing before drug administration should be before antibiotic administration to avoid high level of resistance

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