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Research Article

Multiple Drug Resistance Trends in Uropathogens in Urine of Pregnant Women and HIV Patients in Port Harcourt, Nigeria

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ABSTRACT

This study accessed the multiple drug resistance patterns of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* isolated from the urine of pregnant women and HIV patients in Port Harcourt, Nigeria. A total of two hundred and sixty (260) urine specimen were gotten from pregnant women (130) and HIV patients (130). These specimens were examined for the presence and isolation of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* with the use of cystine lactose electrolyte deficient agar (CLED) media. In addition, antibiotic susceptibility test was carried out and MAR index for each uropathogen was determined. Results showed *Klebsiella pneumoniae* and *Escherichia coli* as the highest occurring uropathogens in pregnant women and HIV patients respectively. All uropathogens from both groups were highly resistant to Amoxicillin and Imipenem and highly susceptible to Ofloxacin and Levofloxacin. Furthermore, there is a high level of multiple drug resistance exhibited by these uropathogens with *Pseudomonas aeruginosa* having 10.0% of the isolate at MAR index of 1. There is therefore the urgent need to control the indiscriminate use of antibiotics, as this will help restore the susceptibility of microbe to antibiotics.

Keywords: Pregnant women, HIV patients, uropathogens, *Pseudomonas aeruginosa*, *Klebsiella* pneumonia, E. *coli.*

Introduction

In spite of all the progress made in understanding the prevalence and multidrug resistance trends associated with uropathogens, these organisms still come up with multiple resistance to antibiotics which pose a major problem in the clinical settings. Uropathogens are microorganisms capable of causing infections in the urinary tract of humans and animals. They are known to occur in pregnancy, especially in third trimesters of pregnancy (Ezugwu *et al*., 2021; Nabbugodi, Gichuhi and Mugo, 2015 and Okonko *et al*., 2009). They are frequently isolated from immune deficiency virus (HIV) patients, despite being on anti-retroviral drugs. These uropathogens have been proven to cause serious public health care infections due to their frequent occurrence in hospitals and community settings where they decrease the quality of a healthy life span (Olowe *et al*., 2015). Uropathogenic *Escherichia coli* (UPEC) has been reported by several authors as the main cause of infections in the urinary tract in both pregnant women and human immuno-deficiency virus (HIV) patients (Johnson *et al*., 2021, Tessema *et al*., 2020; Skrat-Klapaczynska *et al*., 2018 and Ochei *et al*., 2018). Other uropathogens that has been isolated by several other workers include *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus* and many others (Johnson *et al*., 2021 and Ochei *et al*., 2018). Infections in the urinary system is categorized as an important clinical issue in women that needs prompt treatment, mostly during pregnancy. Its significant incident during pregnancy has been analyzed in view of its linked fetal and maternal morbidity and mortality (Cunningham *et al*., 2001). In HIV patients, lowered immunity predisposes uropathogens to colonize the urinary tract.

Antibiotics have been successfully used to treat bacterial infections associated with urinary tract pathogens for many years and have led to management

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of pathogenic organisms easier thereby decreasing the incidence of complications in pregnant women, HIV patience and many others. However, recent proofs suggest that the success attained through antibiotic are threatened by antimicrobial resistance and in most cases, multidrug resistance expressed by these uropathogens especially in hospital where they cause nosocomial infections and usually forms biofilms in urinary catheters. They are also prevalent in community settings where they are dispersed easily.

Globally, there is a growing concern over multidrug resistance exhibited by these uropathogens since the last decade which is a problem that concerns every country irrespective of its level of development since resistant pathogens do not respect borders. Some of the known factors contributing to multidrug resistance included mis-use and over use of antibiotics which are the main tools in the development of drug resistance pathogens. It is said to have eaten into the world to a great depth, especially in sub-Saharan Africa leading to long stay at the hospitals, escalated cost of treatment, complications that can lead to death. If no proper arrangements are taken to end its progress, resistance to antimicrobials is approximated to cause 10 million lives by the next two decades (Oneil, 2016). Despite the warnings, the report given by WHO in 2014, and O'Neill report recently published described the gaps in data sharing, surveillance, and standard methodologies (WHO, 2014). Africa has been recognized one of the regions where there is no good confirmed report to resistance to antimicrobial surveillance systems.
Therefore, the aim of this studies is to determine the multidrug resistance patterns of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* isolated from the urine of Pregnant women on ante-natal care and HIV patients on anti-retroviral drugs in not less than one year, in Port Harcourt, Nigeria.

Materials and Methods

Sample Collection.

Urine samples (130 for each group of participants) was collected from pregnant women and HIV patients. Each participant submitted midstream urine samples with a labelled sterile container which was tightly closed.

The samples were 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 dipped into the urine sample was aseptically and uniformly smeared on a sterile Cysteine Lactose Electrolyte Deficient Agar (CLED) plate, the wire loop was then used to streak the plate of smear to get distinct colonies. This process was repeated for all specimens and incubated for 24-hour at 37°C.

Cultures with up to 200 colonies documented as equivalent to 10^5cfu/ml of urine was noted as being significant at the other hand, cultures presenting less than 200 colonies was recorded as non-significant (Agbagwa & Ifeanacho, 2015).

Identification of Isolates

They isolates were sub cultured to get pure colonies and were stored on agar slants at 4^0C. In-addition, they were identified using their morphological characteristics and further confirmatory tests using standard microbiological techniques which included Gram staining, motility test and biochemical characterization like indole, catalase test, citrate utilization test, coagulase test, oxidase test, Voges – Proskauer test, methyl red test, urease test and sugar fermentation test such as glucose, lactose, mannose, galactose, fructose and maltose fermentation were also applied in identification of these organisms.

Antibiotic susceptibility test

Antibiotic susceptibility testing was performed using Kirby-Bauer's disc diffusion method. The uropathogens were exposed to twelve antibiotics which include, Nalidixic acid (NA)-30ug, Ceftriaxone sulbactam (CRO)-45ug, Levofloxacin (LBC)-5ug, Cefuroxime (CXM)-30ug, Gentamycin (GN)-10ug, Cefexime (ZEM)-5ug, Ofloxacin (OFX)-5ug, Cefotaxime (CTX)-25ug, Imipenem/cilastacin (IMP)-10/10ug, Amoxicillin clavulanate (AUG)-30ug, Nitrofurantoin (NF)-300ug and Ampiclox (ACX)-10ug.

MAR index calculation

The results from antibiotic susceptibility testing were used to calculate the MAR index by dividing the number of antibiotics the uropathogen is resistant to by the total number of antibiotics tested. This is expressed mathematically as follows: $MAR\ index = a/b$ (Osundiya, Oladele and Oduyebo, 2018). A MAR index greater than 0.2 is indicates a high-risk source of antibiotic overuse.

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Results

The prevalence of uropathogens in HIV patients (Figure1) showed Pregnant women had *Klebsiella pneumoniae* (34.04%) as the most occurred uropathogens and *Enterobacter aerogenes* (2.13%) and *Alcaligenes faecalis* (2,13%) as the least occurred uropathogens while *Escherichia coli* (72.22%) as the most occurring uropathogen and *Pseudomonas aeruginosa* and *Proteus* sp. (5.56%) as the least occurred uropathogens in HIV patients.

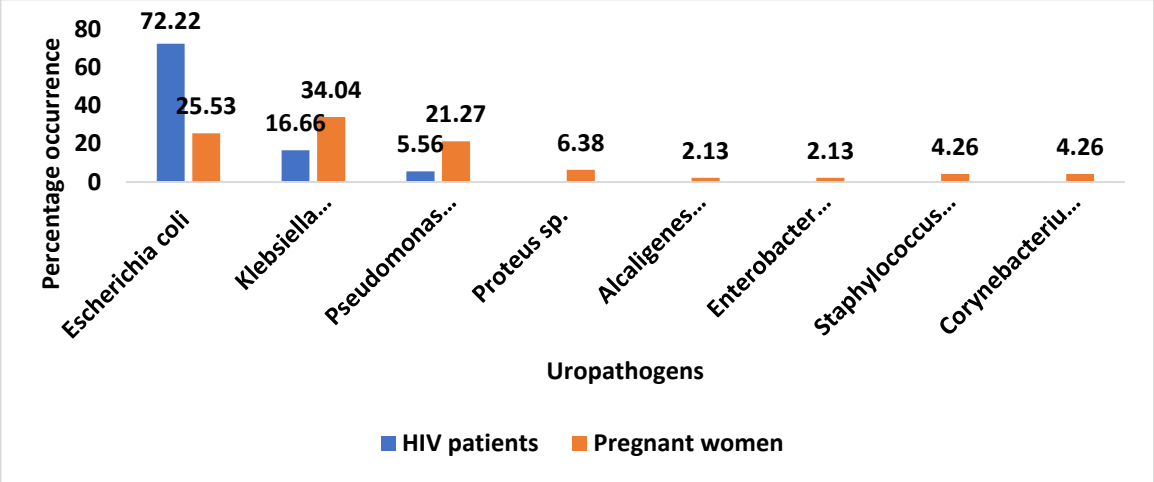

Fig. 1: prevalence of uropathogens in pregnant women and HIV patients

The antibiotic susceptibility test disclosed percentage resistance of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* isolated from urine of HIV patients to antibiotics tested as shown in Figure 2. It revealed that all the uropathogens were 100% resistant to Imipenem/ cilastacin (IMP)-10/10ug and Amoxicillin clavulanate (AUG)-30ug. They were also resistant to many other antibiotics except for Levofloxacin and Ofloxacin where they showed high susceptibility.

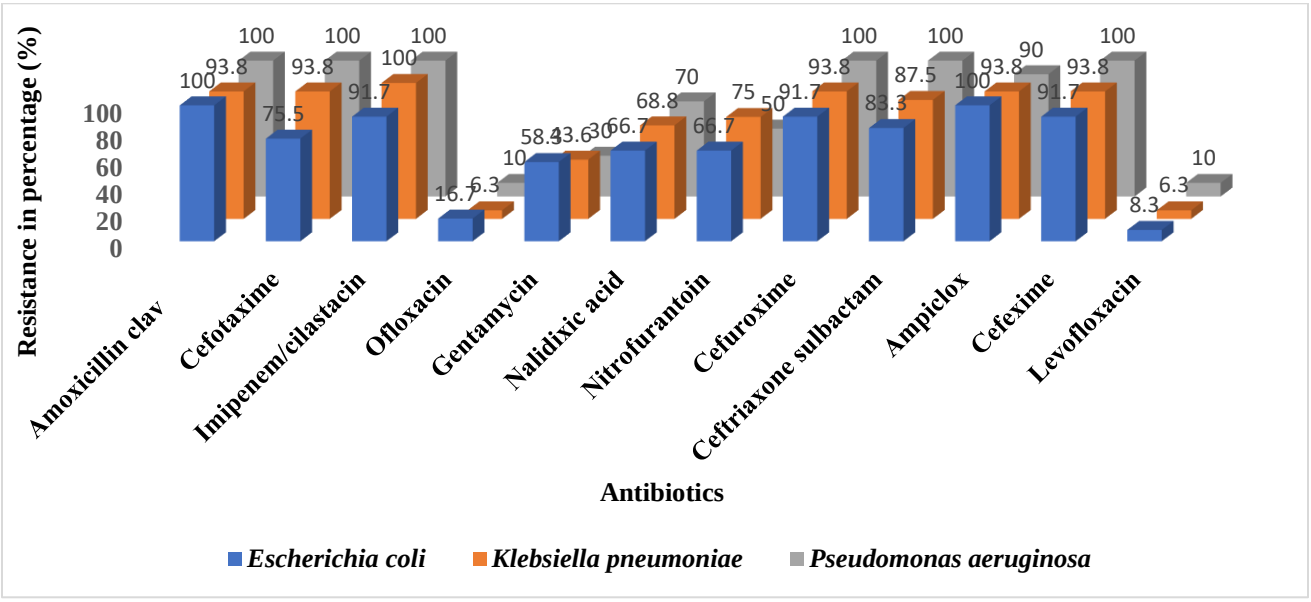

Fig. 2: Percentage resistance of uropathogens isolated from urine of pregnant women to antibiotics tested

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A pattern in percentage resistance of the three uropathogens isolated from urine of pregnant women to antibiotics tested is revealed in Figure 3. The result showed that all uropathogens were resistant to more than six antibiotics and were susceptible to Levofloxacin and Ofloxacin. In addition, only *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were susceptible to Gentamycin.

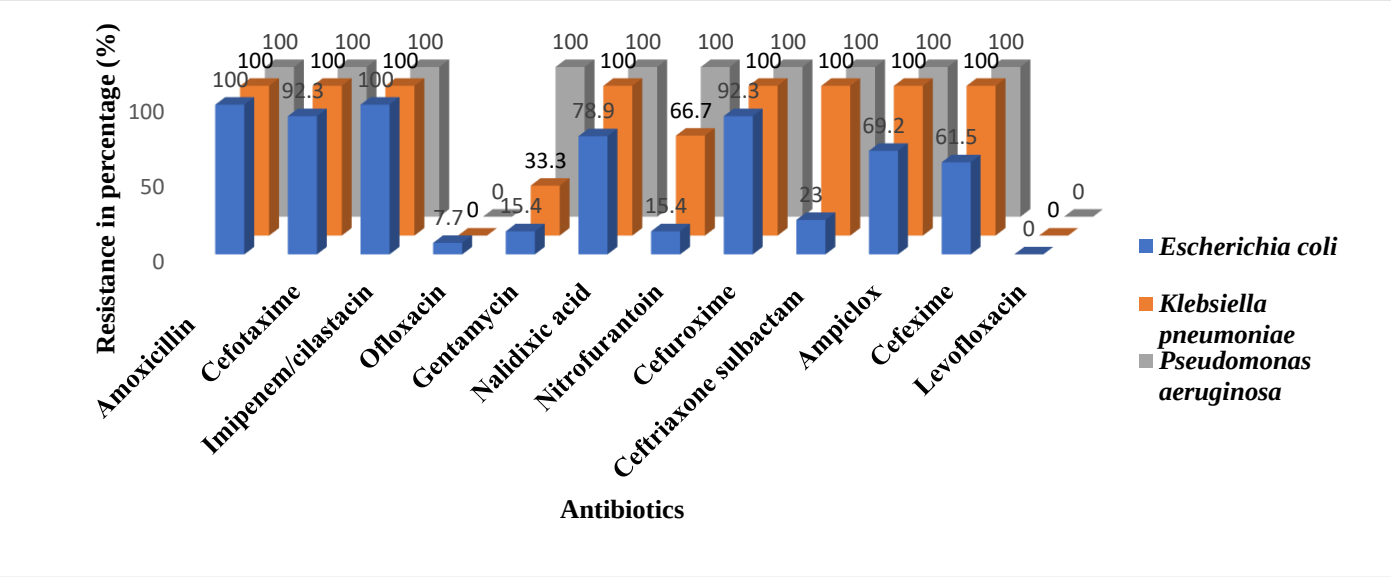

Fig. 3: Percentage resistance of uropathogens isolated from urine of HIV patients to antibiotics tested.

The results for multiple drug resistance index revealed in Figure 4 showed that extreme MAR index ≥ 0.7 in 27.3% and 0.8 in 27.3% of *Pseudomonas aeruginosa* were detected. *Pseudomonas aeruginosa* also revealed mar index of 1 for 9.1% isolates, this means that 9.1% of the isolate was resistant to all antibiotics tested. While the highest MAR index values ≥ 0.8 were detected in 68% of *Klebsiella pneumoniae* as shown in Figure 5. It also revealed *Klebsiella pneumoniae* having MAR index of 0.1 in 5.3% of the isolates.

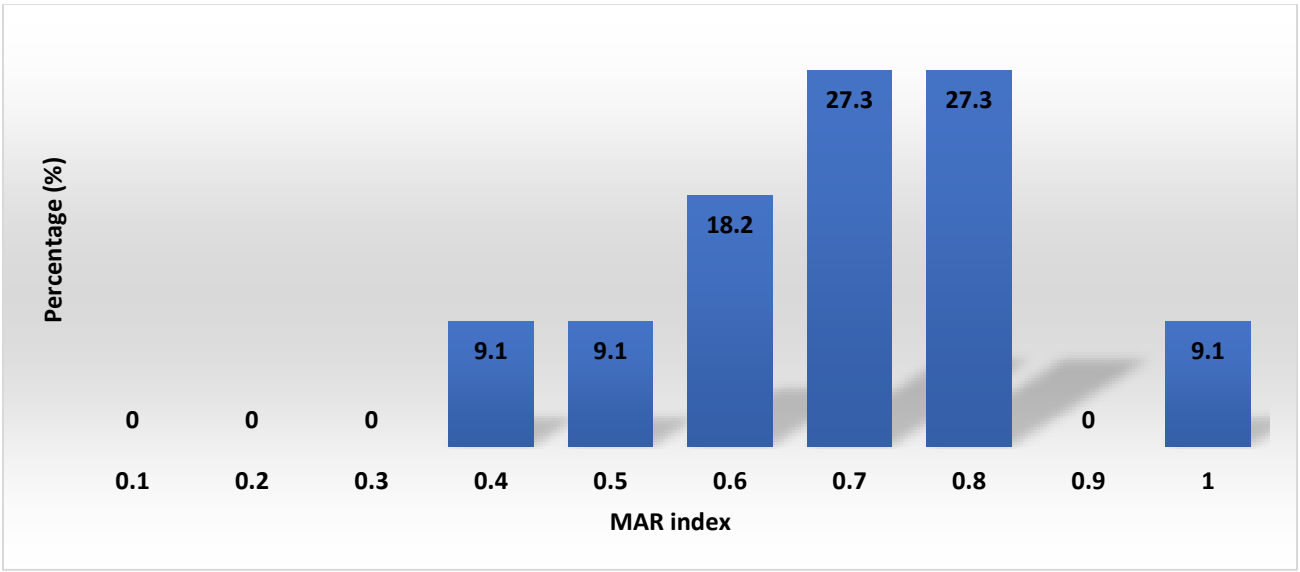

Fig. 4: Distribution of overall MAR index in *Pseudomonas aeruginosa* in pregnant women and HIV patients

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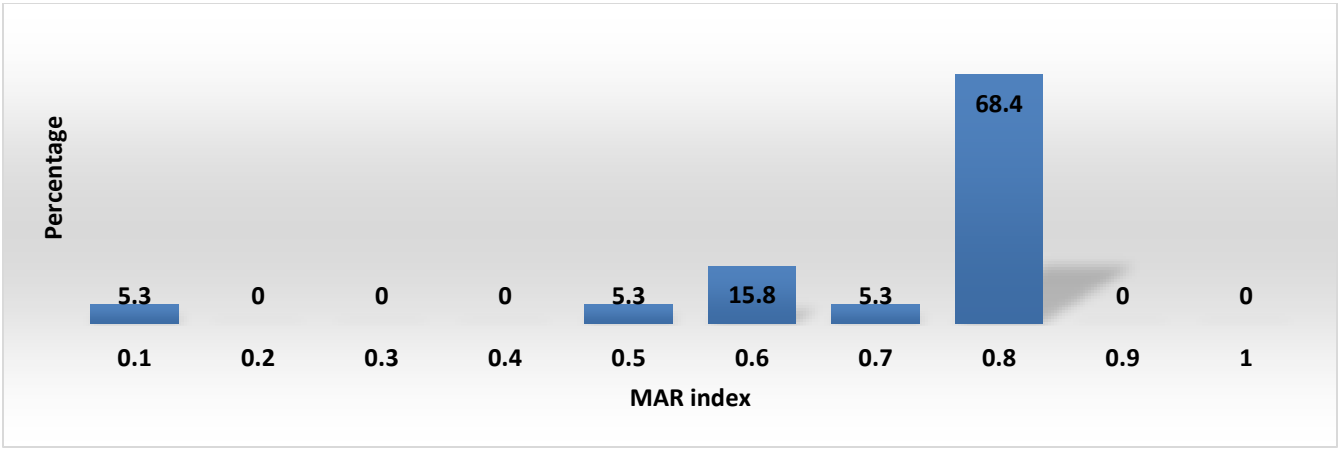

Fig. 5: Distribution of overall MAR index in *Klebsiella pneumoniae* in pregnant women and HIV patients

MAR index values ranging from ≥ 0.6 to 0.8 were detected in *Escherichia coli* as revealed in Figure 6. While Figure 7 showed the combine MAR index distribution of uropathogens from urine of HIV patients and pregnant women**.**

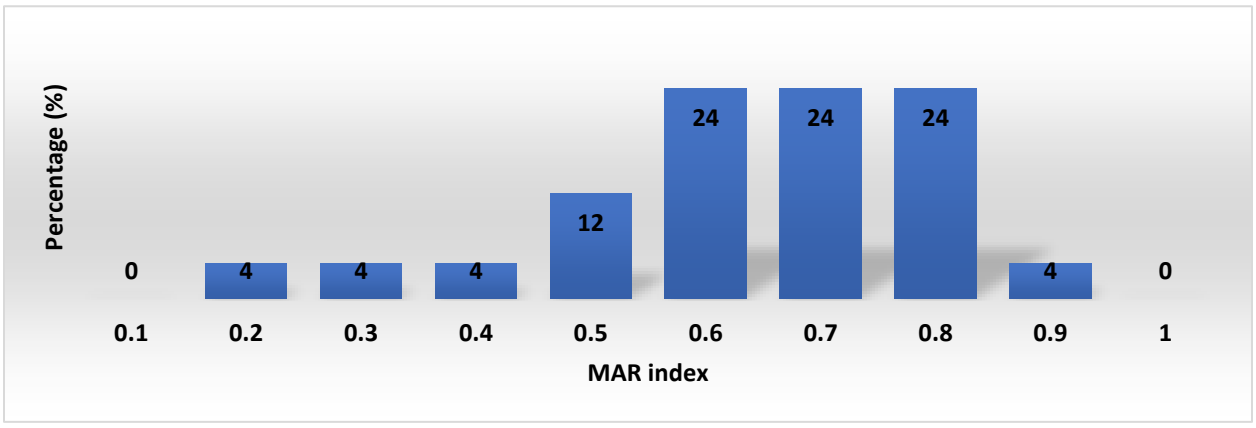

Figure 6: Distribution of overall MAR index in *Escherichia coli* in pregnant women and HIV patients

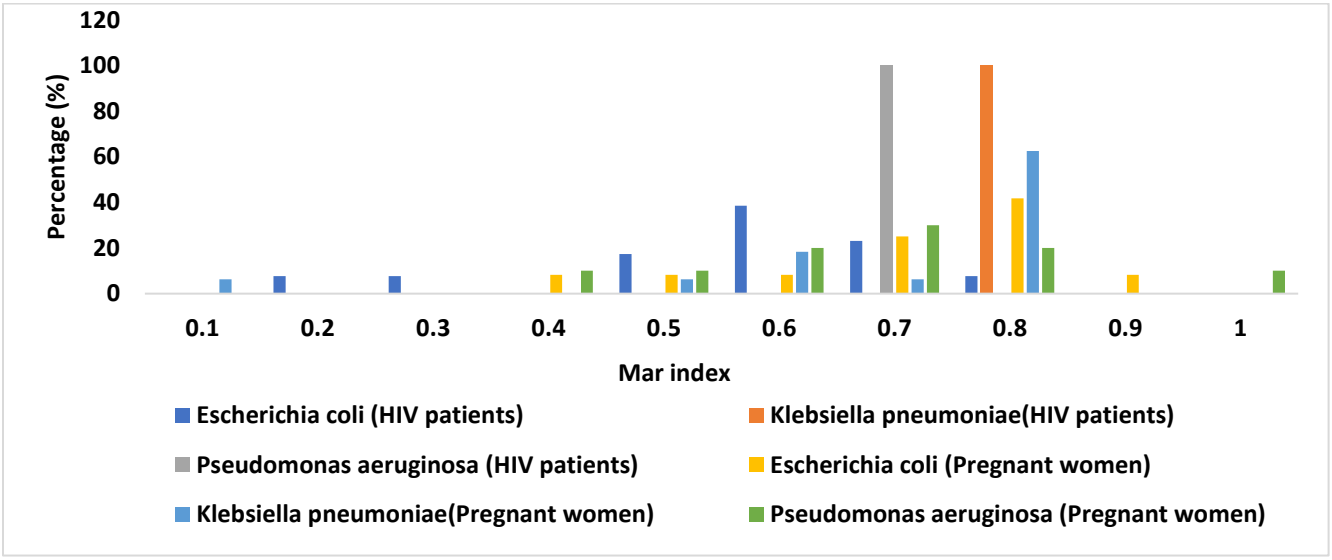

Fig. 7: Combine MAR index distribution of uropathogens from urine of pregnant women and HIV patients

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Discussion

The present study was aimed at determining the multidrug resistance patterns of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* isolated from the urine of Pregnant women and HIV patients. The most prevalent in pregnant women is *Klebsiella pneumoniae*. This is same with the findings carried out by Johnson *et al*., (2021) and Ochei *et al*., (2018), they reported *Klebsiella pneumoniae* (26.5%) as the highest occurring uropathogens.

The most prevalent uropathogen in HIV patients was *Escherichia coli* (72-22%). The result tallied with other author's work including, Tessema *et al*. (2020) and Skrat-Klapaczynska *et al*., (2018). However, this study was inconsistent with findings that revealed *Pseudomonas aeruginosa* (29.7%) as the predominant uropathogens. The higher occurrence of *Escherichia coli* in this study may be due to possession of diverse virulence gene exhibited by this organism, with the most important one being the fimbriae. The difference in the type of uropathogen isolated may be tied to method used in sample collection, personal and environmental hygiene and geographical variations. All the uropathogens from urine of pregnant women and HIV patients (Figure 2 and 3) had a high resistant level for Imipenem/cilastacin, Ampiclox, Amoxicillin/ Clavulanic acid, Cefixime and Cefuroxime indicating the effects of misuse or overuse. This may be as a result of over availability of these drugs over the counters, especially for Amoxicillin/ Clavulanic acid and Ampiclox in Nigeria. The antibiotics, imipenem was widely in use due to their effectiveness in the treatment of resistant bacteria and safety associated with it, this led to its widespread use resulting to resistance to the carbapenems, a beta lactam and broad-spectrum antibiotics which is a main issue to public health (Aurilio *et al*., 2022). The result from pregnant women in this study tallied with research carried out by Johnson *et al*. (2021) and Ejerssa *et al*., (2021) on pregnant women. They reported that all the pathogens were highly resistant to Amoxicillin/Clavulanic acid.

This study is not in conformity with some other study that revealed high susceptibility to Amoxicillin/ clavulanic acid (70%) and imipenem in uropathogens from HIV patients (Assefa *et al*., 2015 and Abongomera *et al*., 2021). All *Pseudomonas aeruginosa*, *K. pneumoniae* and *Escherichia coli* isolates in urine of pregnant women and HIV patients were highly sensitive to Levofloxacin and Ofloxacin.

This corresponds with findings in Nigeria where *Klebsiella pneumoniae* and *Escherichia. coli* was sensitivity to Ofloxacin and Levofloxacin (Onoh *et al*., 2013). There are studies in Nigeria which also pointed out high sensitivity of uropathogens to Ofloxacin (Nwafia *et al*., 2021 and Omoregie and Eghafona, 2009). At the other hand, this did not rhyme with a study where all isolates were resistant to Ofloxacin (Inyang-Etoh *et al*., 2009). The differences might be due to the switch in the trend of antibiotic sensitivity lately noticed in the infections in the urinary tract that is linked with other morbidities which have been ascribed to wide-spread self-medication and non-selective use of antibiotics are more likely with patients suffering from HIV/AIDS (Chaula *et al*., 2017). The susceptibility of the uropathogens to antibiotics tested on them differed based on the bacteria specie. In illustrating this, you observe that the uropathogen, *Escherichia coli* harboured in urine of HIV patients revealed a high susceptibility to Gentamicin, Ofloxacin, Nitrofurantoin, Levofloxacin and Ceftriaxone. They showed 100% resistance to imipenem and Amoxicillin. *Klebsiella pneumoniae* posed resistance to up-to 75% of the antibiotics tested except for Gentamycin, Levofloxacin and Ofloxacin. In a study carried out by some researchers involving *Klebsiella pneumoniae* isolates gotten from clinical samples, results showed resistance of the organism to many of the antibiotics tested which included, Nalidixic acid, imipenem, Ceftriaxone and Cefotaxime (Rastegar *et al*., 2021). This study corresponded to that research in the sense that it revealed *Klebsiella pneumoniae* having 100% resistant to imipenem, and highly resistant to other antibiotics mentioned. Similarly, a high level of resistance to imipenem by *Klebsiella pneumoniae* has been shown in other studies. Some of these studies also disclosed high resistance of *Klebsiella pneumoniae* isolate to Nitrofurantoin and Amoxicillin as seen in this study (Shahi *et al*., 2019 and Amraie *et al*., 2014). Having resistances that are high to most antibiotics experience in *Klebsiella pneumoniae* can be linked to the organism capability to produce enzymes that includes, Extended Spectrum Beta Lactamase (ESBLs) which is a major cause of resistance in some microorganisms. Another reason may be due to its association with health-care associated infections (HAIs) which led to extended use of some of these antibiotics in the hospital, thereby, leading to resistance acquisition to antimicrobial by *Klebsiella pneumoniae* (Nirwati *et al*., 2019).

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Pseudomonas aeruginosa showed high level of resistance to almost all antibiotics including imipenem in the class of carbapenem antibiotics (Figures 2 and 3). The 100% resistance to the imipenem portrayed in this study is quite alarming taking into consideration that the carbapenems are the last line of antibiotics for treating gram-negative bacilli infections. The resistance exhibited may have aroused due to the complex interactions as a result of several mechanisms such as, carbapenemase production, loss of outer membrane porins and over production of efflux system. They were susceptible to Ofloxacin and Levofloxacin which may indicates the proper use of the two antibiotics. The resistant pattern revealed tallied with a study that divulged *Pseudomonas aeruginosa* isolated from urine samples showing 70-100% resistance for gentamycin, Imipenem and other antibiotics tested against them (Nitz De-Melo *et al*., 2021). This resistant trend of *Pseudomonas aeruginosa* can be linked to its intrinsic and its ability to acquire new mechanisms of resistance to many antibiotic groups (Filatraut *et al*., 2006). This result did not agree with a study where it was revealed that *P. aeruginosa* was resistance to levofloxacin due to clonal spread of the organism (Juayang *et al*., 2017). Pregnant women disclosed *Escherichia coli* resistant (100%) to Ampiclox and Amoxicillin, in addition they were highly resistant to Imipenem/cilastacin, Cefexime, Nitrofurantoin and Cefuroxime antibiotics. This agreed with findings of Alshabi and his group, they revealed that pregnant women in their study harboured *Escherichia coli* highly resistant to Nitrofurantoin and Amoxicillin antibiotics (Alshabi *et al*., 2019). There was a high level of susceptibility for *Escherichia coli* for Ofloxacin and Levofloxacin. This corresponded with a study by Muhammed, (2015) on pregnant women who reported high susceptibility of *Escherichia coli* for Ofloxacin.

The multiple drug resistance index (MAR) revealed in figures 4,5,6 and 7 is an important analysis in checking resistance to antibiotics risk factors in health. Uropathogens with MAR index greater than 0.2 confirms the presence of multidrug resistance genes originating from the environment where there is abuse of antibiotics. The MAR index result in this study revealed *Pseudomonas aeruginosa* having a MAR index of 1 in 10% of the isolates (Figure 4). This means that the organism was resistant to all the (12) antibiotics tested against it. It also revealed the *Klebsiella pneumoniae* (Figure 5) had many isolates with a MAR index of 0.8, *Pseudomonas aeruginosa* and *Escherichia coli* (Figure 6) with most isolates on MAR index ranging from 0.6 and 0.8. This is an indicative that individuals involved in this research were exposed to environment of high-risk contamination from an area where there is high use of antibiotics. It also suggested that most conventional drugs used for *Pseudomonas aeruginosa* and other uropathogens studied in this research are not effective as the use to be in the past. Some of these MAR index revealed in this work tallied with results from other researchers, for example, a MAR index of 0.6 to 1 was prevalent in a study carried out on *Escherichia coli* from clinical samples (Ahaji *et al*., 2022). The uropathogens studied in this work revealed a high level of multidrug resistance. One frequently encountered reason for this is the mis-use of antibiotics which refers to prescribing antibiotics for a person without establishing any form of bacterial infection.

In conclusion, uropathogens are still prevalent among HIV patients and pregnant women despite being on anti-retroviral drugs and going for ante-natal care respectively. Even though there was high resistance rate for the three uropathogens, the Levofloxacin and ofloxacin had a good effect on them. However, some resistance that occurred especially to carbapenem was alarming and these isolates may act as a source of infection that can threaten the health of patients. Guidance in the use of antibiotics is still recommended to limit the increased resistance to antimicrobials occurring presently.

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