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Prevalence of ESBL-producing and multi-drug resistant *Escherichia coli* isolated from urine of HIV patients in General Hospital Gboko, Benue State

Terwase, Aondona Jerry [1, 2], Umeh, Ebele U [2], Ichor, Smart Tersagh [2, *] and Gberikon, Grace Mwuese [2]

[1] *Department of Science Laboratory Technology, Fidei Polytechnic Gboko, Benue State, Nigeria.*
[2] *Department of Microbiology, University of Agriculture, Makurdi, Benue State, Nigeria.*

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Abstract

Prevalence of ESBL-producing and multi-drug resistant *Escherichia coli* in HIV patients attending General Hospital Gboko, Benue State was investigated. A total of two hundred and five urine samples were collected from patients in the hospital and inoculated on Cysteine lactose electrolyte deficient (CLED) agar. The plates were incubated at 37 ^{0}C for 24 hours for isolation of bacteria. Bacteria isolates were characterized by Gram staining and biochemical tests. Antibiotic resistance testing was performed using Kirby-Bauer disc diffusion method. The results revealed a prevalence of 23(11.22%) in this group. Sex prevalence of ESBL-producing *Escherichia coli* was observed to be significantly higher in female subjects 20(12.98%) compared to male patients 3(5.88%). All isolates were resistant to Cefuroxime 23(100.0%), Augmentin 23(100.0%), followed by Ceftazidime 22(95.65%), Gentamycin 20(86.96%), Nitrofurantoin 19(82.6%), Ofloxacin 18(78.26%), and Ciprofloxacin 18(78.26%). The least resistance was observed with the antibiotic Cefotaxime 13(56.5%). The presence of ESBL-producing and multi-drug resistant *Escherichia coli* in urine samples of HIV patients indicates an infection which presents a major threat to public health since such infections may be difficult to treat and may subsequently result to death of the patients. Constant periodic surveillance of urinary tract pathogens isolated from HIV patients and non-abuse of antibiotics will help check this ugly trend especially in developing nations like Nigeria.

Keywords: ESBL; MDR; *Escherichia coli*; UTIs; HIV patients

1. Introduction

Antimicrobial resistance has become a major public health challenge today in both developed and developing nations of the world. Drug resistance is a major factor contributing to mortality and morbidity worldwide [1].Urinary tract infections (UTIs) are some of the most common kind of infectious diseases in humans that can affect at least 250 million individuals annually [2]. UTI's can be caused by Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Proteus* species and Gram-positive bacteria like *Enterococcus* species, and *Staphylococcus saprophyticus* [3].In both community and hospital settings *Escherichia coli* and *Klebsiella pneumonia* are the most predominant Gram-negative bacteria responsible for urinary tract infections globally occurring with both symptoms and in some cases asymptomatic[4].

Human Immunodeficiency Virus (HIV) is the virus that causes immunodeficiency syndrome that result to the progressive breakdown of the human immune system. This break down of the immune system allows life threatening opportunistic infections to thrive. If left untreated, HIV infection will eventually develop to AIDS and the infected individual usually die of opportunistic infections or malignancies associated with immune system failure [5].Globally, it is estimated that Thirty-four (34) million people are living with human immunodeficiency virus (HIV) and annually, about 1.8 million people die of HIV/AIDS related disease and 1.9 million persons are infected in Sub-Saharan Africa [6].

* Corresponding author: Ichor Tersagh
Department of Microbiology, University of Agriculture, Makurdi, Benue State, Nigeria.

The genitourinary system of people living with HIV/AIDS is affected by different diseases and such people are highly vulnerable to bacterial infections due to low CD4 count and high viral load. Urinary tract infections are one of the most common bacterial infections and the cause of morbidity and hospitalization in HIV positive individuals [7].

Several antibiotics are used in treatment of UTIs worldwide. The choice of antibiotic to be used is mostly determined by the availability and cost. These and several other factors may also contribute to the widespread use and subsequently microbial resistance of a particular antimicrobial agent. For instance, beta-lactam antibiotics are widely used in Nigeria against aerobic Gram-negative bacilli infections and these results to a selective pressure on the beta-lactam drugs which subsequently results in strains developing extended spectrum beta-lactamases (ESBLs) enzymes [11]. ESBLs are enzymes capable of hydrolyzing extended spectrum/third generation Cephalosporin. These enzymes are also produced by *Escherichia coli* which enables the pathogen to resist Penicillin, Cephalosporin's (i.e. First, Second and Third generation), and Monobactams. ESBL *Escherichia coli* pathogens remain susceptible to Carbepenems and may or may not be susceptible to beta-lactam/beta-lactamase inhibitor combinations [8].

There is an alarming increase in cases of antibiotic resistance especially those use in the management of UTIs worldwide and this has potential for doing more harm in developing countries like Nigeria since most patients cannot afford/access quality antibiotics. The situation is compounded by factors such as high poverty level, ignorance, poor hygiene practices as well as high proliferation of fake drugs in the markets [9]. Currently, there is a dearth of information on the status of Urinary tract infections caused by ESBL-producing multi-drug resistant *Escherichia coli* isolated from urine samples of HIV patients in Gboko. The burden of drug resistance coupled with inadequate data on the subject matter in this area underscores the importance of this study.

Aim of the Study

The aim of the study is to determine the prevalence of ESBL-producing and multi-drug resistant *Escherichia coli* in urine samples of HIV patients attending General Hospital Gboko.

Objectives of the Study

The specific objectives of the study include:

- To isolate *Escherichia coli* from urine of HIV patients attending General Hospital Gboko using conventional culture approach and determine its antibiotic resistance profile.
- To confirm ESBL production in *Escherichia coli* isolates using Double disc synergy test and determine its distribution according to Age and Sex in urine of HIV patients.

This study is significant because, it will unravel the status of UTIs in HIV patients caused by ESBL producing multi-drug resistant (*Escherichia coli)* bacteria in this part of the world. The study will also suggest possible ways of managing such infections and contribute significantly to the already existing literature on the subject matter. It will also serve as baseline data for multi-drug resistant *E. coli* in HIV patients in Gboko and address the problem of the existing gap on data from studies in this regard.

The research work covers prevalence of multi-drug resistant *Escherichia coli* isolated from urine samples of patients attending General Hospital Gboko. It does not include any other bacteria that may be responsible for urinary tract infection in this group of persons under study.

2. Materials and methods

2.1. Study area

The study was carried out in Gboko General Hospital which is located in the South-western part of Gboko town. The town is the headquarters of the Tiv traditional council, and the headquarters of Gboko local government area of Benue state, Nigeria. Gboko is located on latitude 18/30 and longitude 7/45. Gboko has a population of nearly 500,000 inhabitants who are mostly civil servants, farmers and traders. Most of these people receive medical care in General Hospital Gboko.

2.2. Materials Sterilization

Glass wares used were washed with detergents using tap water, and sterilized by autoclaving at 121^{0}C for 15 minutes after drying. Sample containers and swab sticks were bought sterile and used once for each specimen. Hand gloves were bought sterile and disposed immediately after single use.

2.3. Sample Collection

Cross-sectional study was conducted on HIV patients attending General Hospital Gboko, Benue State. Ethical approval was sought and obtained from the Benue State Ministry of Health and Human Services Makurdi, for sample collection in the Hospital.

Participants were properly informed about the purpose and requirements of the research and patients who consented were advised to collect Ten (10) ml of their midstream urine into the sample container after which the lid of the container was replaced and taken to the laboratory for analysis. The result of this study was confidential and only available to interested participants on request to assist health-care providers in treatment. Urine samples from two hundred and five (205) HIV patients attending General Hospital, Gboko were collected between August and September, 2017.

2.4. Preparation of Culture Media

Media for analysis, CLED agar, Simmons Citrate agar, Mueller-Hinton agar, Trypton broth and MR-VP broth (TM media, India) were prepared according to manufacturer's specification and sterilized accordingly.

2.5. Isolation of *Escherichia coli* from urine samples

Urine samples were cultured on CLED agar by streaking, the plates were incubated at 37 ^{0}C for 24 hours, after which suspected *E. coli* colonies were sub-cultured on fresh plates to isolate pure colonies.

2.6. Characterization of Isolates

2.6.1. Gram staining

Suspected *Escherichia coli* colonies were Gram stained as described by Cheesbrough [10] to determine their Gram reaction.

2.6.2. Biochemical tests

Further characterization of the isolates was achieved by (i).Indole test (ii). Methyl red test (iii).Voges-Proskauer test and (iv). Citrate tests as described by Cheesbrough [10].

2.6.3. Antimicrobial Susceptibility Tests on Escherichia coli

Kirby-Bauer disc diffusion method described by Nwosu *et al.* [11], was used for susceptibility testing. Isolated colonies of *Escherichia coli* were inoculated into peptone water and incubated at 37 ^{0}C for 2-6 hours. The turbidity was adjusted to 0.5 McFarlands standard and lawn culture was prepared on Muller-Hinton agar using sterile swab sticks. Antibiotic sensitivity discs were placed on the culture plates using a sterile forceps. The plates were covered, inverted and incubated at 37 ^{0}C for 24 hours. A meter rule was used to measure the zones of inhibition to the nearest millimeter.

2.6.4. ESBL Confirmation by Double Disc Synergy Test

The method of Nwosu *et al.* [11], and Nwankwo *et al.* [12], which conform to Clinical Laboratory Standards Institute (CLSI), was adopted for ESBL confirmation. Isolated colonies were inoculated into peptone water and incubated at 37 ^{0}C for 2-6 hours. The turbidity was adjusted to 0.5 McFarland's standard and lawn culture was prepared on Muller-Hinton agar using sterile swab stick. Augmentin (Amoxycillin/Clavulanic acid) disc (20/10 µg) was placed in the center of the plate, a disc of Cefotaxime (30 µg) and Ceftazidime (30 µg), was placed center to center at a distance of 15 mm to centrally placed Augmentin (Amoxicillin/Clavulanic acid) disc. The plates were incubated overnight at 37 ^{0}C. Enhanced zone of inhibition between any of the beta-lactam discs and the center disc was recorded. Increased zone of inhibition between any of the third generation cephalosporin antibiotic discs and the Augmentin (Amoxicillin/Clavulanic acid) disc relative to the corresponding disc without the beta-lactamase inhibitor was a confirmation of ESBL production.

3. Results

Prevalence of ESBL-producing and multi-drug resistant *Escherichia coli* isolated from urine samples of HIV patients attending General Hospital Gboko, Benue State was investigated. Two hundred and five (205) urine samples were collected from HIV patients between the months of August and September 2017 after obtaining ethical clearance from the hospital authorities.

Table 1 Prevalence of ESBL-producing and multi-drug resistant *Escherichia coli* isolated from urine of HIV patients in General Hospital, Gboko

Status	Number	Percentage (%)
Positive	23	(11.22%)
Negative	182	(88.78%)
Total	205	(100%)

Table 2 Sex distribution of ESBL-producing and multi-drug resistant *Escherichia coli* isolated from urine of HIV patients in General Hospital, Gboko

Sex	Number sampled	Positive (%)	P-Value
Male	51	3(5.88%)	0.0446
Female	154	20(12.98%)	
Total	205	23(11.22%)	

χ =0.996, d.f=1

Table 3 Age distribution of ESBL-producing and multi-drug resistant *Escherichia coli* isolated from urine of HIV patients in General Hospital, Gboko

Age (Years)	Number Sampled	Positive (%)	P-Value
1-10	10	0(0.0%)	0.067
11-20	13	2(15.4%)	
21-30	62	7(11.3%)	
31-40	62	7(11.3%)	
41-50	43	5(11.63%)	
51-60	14	2(14.3%)	
61 and Above	1	0(0.0%)	
Total	205	23(11.22%)	

χ =32.53, d.f=5

Table 1 shows the prevalence of multi-drug resistant *Escherichia coli* isolated from urine samples of HIV patients attending General Hospital Gboko. Twenty-three 23(11.22%) ESBL-producing and multi-drug isolates were obtained from different patients in this study while 182(88.78%) patients tested negative. Table 2 shows the sex distribution of ESBL-producing and multi-drug resistant *E. coli* in urine of HIV patients in this study. Sex distribution of ESBL-producing and MDR *E. coli* in female HIV patients was significantly higher 20(12.98%) compared to male HIV patients 3(5.88%) (P=0.0446). Table 3 shows the age distribution of ESBL-producing and multi-drug resistant *E. coli* in urine samples of HIV patients. In this study, the age group 1-10years showed a prevalence of 0(0.0%), for the age group11-20years prevalence was 2(15.4%), while age groups 21-30years and 31-40years both had infection prevalence of 7(11.3%).

ESBL-producing and multi-drug resistant *E. coli* infection prevalence in the age group 41-50years was 5(11.63%), 51-60 years 2(14.3%) and the age group 61years and above showed zero prevalence 0(0.0%).Figure one (1) shows antibiotic resistance profile of ESBL-producing and multi-drug resistant *Escherichia coli* isolated from urine of HIV patients in this study. All *E. coli* isolates were resistant to Cefuroxime 23(100.0%) and Augmentin 23(100.0%), followed by Ceftazidime 22(95.65%), Gentamycin 20(86.96%), Nitrofurantoin 19(82.6%), Ofloxacin 18(78.26%), Ciprofloxacin 18(78.26%), isolates showed minimal resistance to Cefotaxime 13(56.5%).

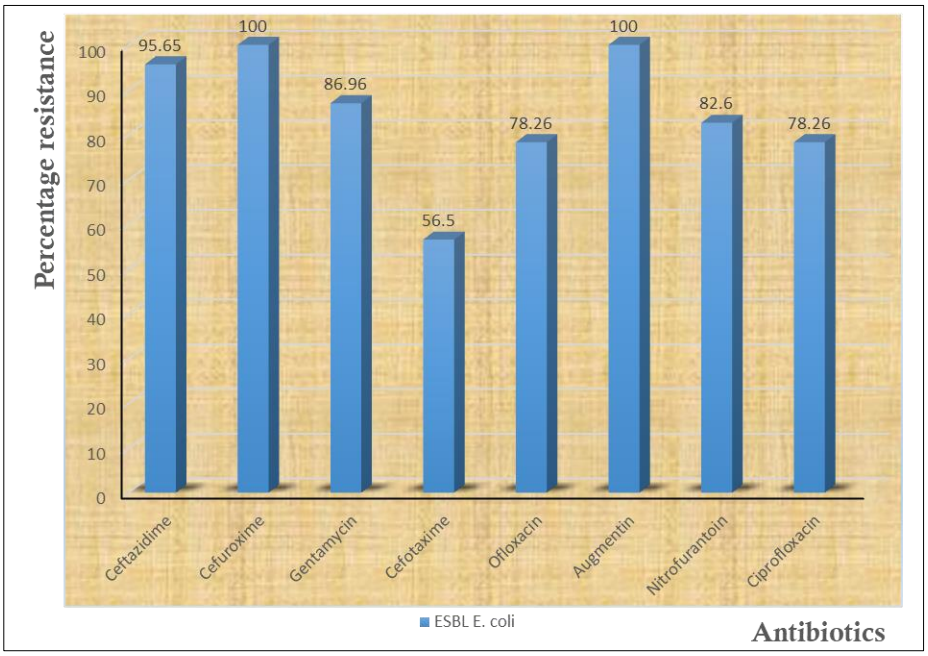

Figure 1 Antibiotic resistance profile of ESBL-producing *Escherichia coli* isolated from HIV patients attending General Hospital, Gboko

4. Discussion

This study was conducted to investigate the prevalence of ESBL-producing and multi-drug resistant *Escherichia coli* isolated from urine of HIV patients in General Hospital Gboko, Benue State. The distribution of ESBL-producing and multi-drug resistant *Escherichia coli* in this study was observed to be 23(11.22%) while 182(88.78%) patients tested negative to the pathogen. In this study, urinary tract infections were found to be significantly higher in female subjects 20(12.98%) compared to male subjects3(5.88%). Prevalence of ESBL-producing *Escherichia coli* UTI in female HIV patients was two times the number in male HIV patients. Age distribution of ESBL-producing and multidrug resistant *E. coli* showed no statistically significant prevalence among the age groups investigated. However, the age group 11-20 years had the highest prevalence, followed by the age group 51-60 years and 41-50years. These are mostly patients whose compromised immunity arising from HIV infection may also be waning due to advancement in age.

The presence of ESBL-producing and multi-drug resistant *Escherichia coli* in urine of HIV patients attending General hospital Gboko, Benue State presents a serious threat to human health in this part of the world. This is further confirmed by our result of Antibiotic resistance profile conducted on *Escherichia coli* isolates in this study which found that all isolates were ESBL-positive. Isolates were all resistant to Cefuroxime 23(100.0%) and Augmentin 23(100.0%). Resistance to Ceftazidime in this study was observed to 22(95.65%), followed by Gentamycin 20(86.96%), Nitrofurantoin 19(82.6%), Ofloxacin 18(78.26%), Ciprofloxacin 18(78.26%) and the least resistance was observed with the antibiotic Cefotaxime 13(56.5%).

This study results agrees with the findings of Debalke *et al.* [6] who reported an increased prevalence of UTI in HIV/AIDS patients with prevalence rate of 6.3% - 41% around the world. Similarly, Niranjan and Malini, [3] reported higher resistance rates of uropathogenic *E. coli* to various antibiotics such as Beta-lactams (57.4%), Co-trimoxazole (48.5%), Quinolones (74.5%), Gentamicin (58.2%), Amikacin (33.4%), Cefuroxime (56%)and Nalidixic acid (77.7%).

Our study is in line with previous work of Kemajou *et al.* [13]who reported a total of 165 bacteria were isolated and identified from the urinary tract of HIV seropositive and negative patients, out of which *Staphylococcus aureus* had the

highest percentage of occurrence 49(29.7%), followed by *Escherichia coli* 47(28.5%), *Pseudomonas aeruginosa* 46 (27.9%) and *Klebsiella pneumoniae* 23(13.9%). The study further revealed that, out of 111 bacterial isolates that were multidrug resistant, 103 (92.8%) were isolated from HIV Seropositive individuals while 8(7.2%) were isolated HIV seronegative individuals. Over all, *Staphylococcus aureus* recorded the highest number of multidrug resistant bacteria 36 (32.4%), followed by *Pseudomonas aeruginosa*34 (30.6%).

Our study agrees with the work of Olorumola *et al.* [14], which reported a higher prevalence of UTIs in female patients 59.1 %in Ile-Ife Nigeria than male patients 40.9% with a wide range of resistance of *E. coli* isolates to commonly used antibiotics in the environment such as Amoxicillin, Gentamycin, Cotrimoxazole, Augmentin, Ciprofloxacin, Norfloxacin, Streptomycin and Nalidixic acid.

All *Escherichia coli* isolates in our study were resistant to Augmentin (Amoxicillin/Clavulanic acid, 20/10ug) 23(100.0%). These also reflected in the ESBL confirmatory test as there was no much enhanced zones of inhibition especially with the antibiotics that showed low sensitivity to the pathogens. This finding agrees with the works of Afridi and Farooqi, [15], which reported that Amoxicillin/Clavulanic acid had poor antimicrobial activity against all ESBL producing *Enterobacteriaceae*. The study further stated that Cefoperazone/Sulbactam combination exhibited the best antimicrobial activity against ESBL producing *Enterobacteriaceae* followed by Piperacillin/Tazobactam combination. Similarly, Walkty, [8], stated that ESBL-producing *E. coli* typically demonstrated resistance to Penicillins, Cephalosporins including; first, second and third generation and Monobactams. The report clearly stated that, ESBL *E. coli* remain susceptible to Carbapenems and may or may not be susceptible to beta-lactam/beta-lactamase inhibitor combinations such as Amoxicillin/Clavulanic acid.

Urinary tract infections due to ESBL-producing and Multi-drug resistant (MDR) *Escherichia coli* increases the cost of treatment and is responsible for pro-longed hospitalization, morbidity and mortality in HIV patients especially in developing nations of the world. The inappropriate use of antimicrobial agents, improper hygiene, prolonged hospitalization and immune compromise as observed with HIV patients who do not follow the antiretroviral therapy properly may promote antimicrobial resistance. In developing countries like Nigeria the problem is further elevated by the use of poor quality or in some cases fake antimicrobials in treatment of urinary tract infections [16].

5. Conclusion

Urinary tract infections in HIV patients caused by ESBL-producing and Multi-drug resistant *Escherichia coli* are becoming a global threat nowadays. These infections accounts for a large proportion of the opportunistic infections experienced by HIV positive persons worldwide. The widespread use of substandard antibiotics as well as the outright abuse of drugs in community and hospital settings has culminated to widespread drug resistance. Treatment of UTIs caused by *Escherichia coli* is becoming a public health issue nowadays because isolated pathogens exhibits high percentage resistance to common antibiotics. These multi-drug resistant (MDR) bacteria pathogens continuously live and multiply in HIV positive patients and become an important source of infection in the community.

We suggest that constant periodic surveillance of urinary tract pathogens isolated from HIV patients in hospitals and community settings will help check the trend of multi-drug resistant bacteria that are responsible for urinary tract infections. Also, patients should avoid buying antibiotics without prescription by a medical expert, culture and antibiotic sensitivity tests should also be the basis for administration of antibiotics in case of an established infection.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict or competing interest.

Statement of informed consent

All procedures performed during this study involving human participants were in accordance with the ethical standards of the Benue State Ministry of Health and Human Services and with 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Author's Contributions

This work was carried out in collaboration between all authors. Author Terwase, Aondona Jerry designed the project in collaboration with the co-authors and wrote out the article to this present standard. He planned and led the execution of the research, E.U Umeh analyzed data and effected all the corrections. Author Ichor, T. and G. M Gberikon assisted in the design, execution and also contributed in the literature search, drafting of the article and statistical analysis. Final manuscript was read and approved by all the authors.

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