

Incidence and antibiotic susceptibility profile of uropathogenic *Escherichia coli* positive for extended spectrum β -lactamase among HIV/AIDS patients in Awka metropolis, Nigeria

Chikaodili J. Nwokolo¹, Malachy C. Ugwu^{1*}, Chika P. Ejikeugwu², Ifeanyichukwu R. Iroha², Charles O. Esimone¹

¹Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria

²Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakilikii, Nigeria

Received: October 2021, Accepted: March 2022

ABSTRACT

Background and Objectives: This study investigated the incidence and antibiotic susceptibility profile of extended spectrum β -lactamase (ESBL) producing uropathogenic *Escherichia coli* recovered from HIV/AIDS patients in Awka metropolis, Nigeria.

Materials and Methods: A total of 363 urine samples were bacteriologically analyzed for the isolation of *E. coli* isolates which were further characterized using standard microbiology techniques. The isolated uropathogenic *E. coli* was tested for susceptibility to a range of clinically important antibiotics using the modified disk diffusion technique. All *E. coli* isolates were phenotypically screened for ESBL production using the combined disk technique, and strains which were positive were further confirmed for the presence of ESBL genes using PCR technique.

Results: A total 160 (44.1%) non-duplicate isolates were bacteriologically confirmed to be uropathogenic *E. coli* (UPEC). The *E. coli* isolates showed reduced susceptibility to important antibiotics including ceftazidime (76.88%), cefuroxime (77.5%), cefixime (61.88%), amoxicillin-clavulanic (32.5%) and ciprofloxacin (34.38%). Twenty-seven of the UPEC isolates were phenotypically confirmed to be ESBL producers. PCR test confirmed some important genes mediating ESBL production in Gram negative bacteria including *bla*_{TEM} (5.0%) and *bla*_{CTX-M-15} (6.9%) genes.

Conclusion: We report a high prevalence of ESBL producers among HIV/AIDS patients in Awka, Nigeria. This result is important as antibiotic resistance (ABR) particularly those mediated by multidrug resistant bacteria as reported in this current study could complicate treatment outcome, worsen the individual's health, and even increase cost of treatment and hospitalization. It is therefore important to lookout for ESBL positive UPEC amongst HIV/AIDS patients in Nigeria.

Keywords: HIV; Drug resistance; Uropathogenic *Escherichia coli*; Antimicrobial resistance; Extended-spectrum β -lactamase

INTRODUCTION

Antibiotic resistance particularly those caused by multidrug resistant bacteria is a global health prob-

lem that negatively affects the health sector and incapacitates our ability to effectively threat and manage infectious diseases caused by these pathogens. Extended-spectrum β -lactamases (ESBLs) are β -lact-

*Corresponding author: Malachy C. Ugwu, Ph.D, Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria. Tel: +2348039460570 Email: mc.ugwu@unizik.edu.ng

amase enzymes that hydrolyze extended - spectrum cephalosporins with an oxyimino side chain but are inhibited by clavulanic acid. They occur mostly in Gram-negative bacteria including members of the *Enterobacteriaceae* family (1), and they are mediate multidrug resistance in organisms harbouring mobile genetic elements or genes for their production. Antibiotic resistance affects human health, animal health, the environment and food security. It is a common occurrence among people with urinary tract infections and other hospital and community acquired infections.

Urinary tract infections (UTIs) are among the commonest human bacterial infections occurring both in the community and hospital settings mostly in developing countries with a high rate of casualty and financial implications (2-3). Clinically, UTI occurs when the number of microorganisms ($> 10^5$ cells per milliliter) in urine is detected in properly collected mid-stream clean catch urine samples (2, 4). UTI's are caused by a variety of bacterial pathogens but mostly by the members of the *Enterobacteriaceae* family including *Escherichia coli*, *Klebsiella* and *Proteus* (3, 5-6). Individuals that are HIV positive are more inclined to getting a UTI because their immunity is already suppressed due to the unique pathogenesis of the virus (HIV) which decreases the CD4⁺ cells of HIV infected individuals (7).

Escherichia coli accounts for about 75-95% of all UTIs because *E. coli* is a normal flora of the GIT and can easily migrate to the urethra from the perineum when proper personal hygiene is not imbibed. Antibiotics particularly antibacterial drugs such as nitrofurantoin, aminoglycosides and fluoroquinolones are mostly the drugs of choice for most UTI's. However, ESBL mediated resistance has been increasingly reported among HIV patients with UTI caused by *E. coli* (8). The emergence of ESBL-producing uropathogenic *E. coli* (UPEC) is of public health concern and a challenge to HIV patient management (9).

Patients with infections caused by ESBL-producing *E. coli* may experience poor prognosis and high morbidity/mortality as well as high cost of treatment if proper medical care is not administered. This could result from delays in initiating effective antimicrobial therapy as well as lack of availability of limited antibiotic options because the isolates often co-express resistance to other classes of antibiotics due to their multidrug resistance nature (1, 10-11). The inability of hospital laboratories in Nigeria, Africa, and other

parts of the world to initiate and carryout proper antimicrobial susceptibility testing (AST) outside their routing AST protocols, that will spot and detect multidrug resistant microbes including ESBL-producing UPEC strains may also be a contributing factor associated with the poor prognosis of HIV patients having underlying or secondary UTI's. There is paucity of information on the frequency and antibiotic susceptibility profiles of ESBL-producing UPEC among HIV/AIDS patients in Awka, Nigeria. Therefore, this study aimed at providing some preliminary epidemiological data that will help to guide therapy amongst HIV positive patients who may be infected with a UPEC strain as a secondary infection.

MATERIALS AND METHODS

Isolation and identification. Clean catch urine samples were collected from HIV positive patients who had UTI and attended a state-owned university teaching hospital in Awka metropolis, Nigeria for medical care. Verbal informed consent was obtained from all patients prior to specimen collection and the study was conducted after obtaining ethical statement (COOUTH/CMAC/ETH.C/VO1.1/0044) from the ethical committee of the said hospital. Non-duplicate urine samples were collected in clean sample collection bottles and transported to the laboratory for bacteriological analysis. All samples were initially cultured on MacConkey agar and cysteine lactose electrolyte deficient (CLED) and incubated overnight at 37°C for the isolation of suspect uropathogenic *E. coli*. Plates showing growth were subsequently cultured on freshly prepared MacConkey agar for the isolation of pure colonies for next step of the experiment. *E. coli* colonies were confirmed using Gram negative staining, indole test, and lactose fermentation on triple sugar iron agar (TSIA) tubes.

Antibiotic susceptibility testing. Antibiotic susceptibility testing was performed using cells of *E. coli* that were equivalent to 0.5 McFarland turbidity standards. The modified Kirby-Bauer's Disk diffusion technique was used for determining the susceptibility profile of the *E. coli* isolates to some clinically important antibiotics including ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), cefixime (5 µg), ofloxacin (5 µg), augmentin (30 µg), nitrofurantoin (300 µg) and ciprofloxacin (5 µg). All suscepti-

bility test disks were purchased from Abtek (Abtek, UK). Each isolate was swabbed on freshly prepared Mueller Hinton (MH) agar plates (Oxoid, UK) and susceptibility test disks were aseptically placed at 25 mm apart using forceps. The MH agar plates were incubated at 37°C for 18-24 hr. The inhibition zone diameters (IZD) produced were measured, recorded, and interpreted by comparing the individual zones to the standard antibiotic breakpoints provided by the guidelines of the Clinical Laboratory Standard Institute (CLSI). The individual IZDs were recorded as percentage susceptible or resistant (12, 13).

Screening and phenotypic detection of ESBL producers. The *E. coli* isolates were screened for ESBL production by first checking their susceptibility against a range of third generation cephalosporins including ceftazidime (30 µg), cefotaxime (30 µg), and cefpodoxime 30 µg. *E. coli* isolates that showed reduced susceptibility to any of these cephalosporins as per the recommended antibiotic breakpoints for resistance to third generation cephalosporins (13) were subjected to further testing using combined disk technique for the phenotypic confirmation of ESBL production. These isolates were aseptically swabbed on the surface of Mueller Hinton (MH) agar plates, and a combination disk of amoxicillin-clavulanic acid (AMC) [20/10 µg] was placed at the center of the MH agar plate and cefotaxime (CTX, 30 µg) and ceftazidime (CAZ, 30 µg) were placed on either side of the central disk (AMC-20/10 µg) at 15 mm apart. The plates were incubated for 18-24 h at 37°C. After incubation, a ≥5 mm increase in zone diameter for either of the cephalosporins (CAZ and CTX) tested in combination with AMC (20/10 µg) compared to its zone when tested alone confirms ESBL production in the test *E. coli* isolates phenotypically (14).

DNA extraction and PCR. The total DNA of ESBL-producing *E. coli* isolates in this study was extracted using the Enzo DNA extraction kit (Enzo Life Sciences, USA) according to the manufacturer's guidelines. The extracted DNA samples were stored at -20°C for the PCR amplification of ESBL genes. The specific sequence of primers and PCR conditions for the PCR experiment is shown in Table 1. The PCR products were separated on 1.5% agarose gel electrophoresis and visualized with 0.5 µg/ml ethidium bromide under ultraviolet transilluminator (BIO-RAD, UK). A 100 bp ladder was used as the molecular

marker. The PCR amplification was performed using 4 µl of sample DNA in a total volume of 12.5 µL reaction mixture with amplification conditions (22).

Determination of multiple antibiotic resistance index (MARI). Multiple antibiotic resistance index (MARI) was determined according to a previous methodology (15). MARI was evaluated by dividing the number of resistant isolates to the total number of antibiotics for which they were evaluated for susceptibility. To determine resistant profile of these resistant isolates, MARI was evaluated using the formula: $MARI = a/b$, where "a" is the number of antibiotics to which the resistant bacteria was resistant to and "b" is the total number of antibiotics to which the resistant bacteria has been evaluated for.

RESULTS

A total of 363 mid-stream urine samples from HIV positive patients who attended a state-owned hospital were bacteriologically analyzed for the isolation of uropathogenic *E. coli* isolates that were investigated further for ESBL production using both phenotypic and genotypic (molecular) technique. One hundred and sixty (160) isolates of *E. coli* were recovered from the urine mid-stream urine samples and used for the next steps of the experiment.

Results of antibiotic susceptibility testing of the uropathogens. The antibiotic resistance pattern of the UPEC isolates was studied and it showed a 76.88% resistance to ceftazidime, 77.5% resistance to cefuroxime, 61.88% resistance to cefixime, 6.25% resistance to gentamicin, 6.88% resistance to ofloxacin, 32.5% resistance to augmentin, 0.63% resistance to nitrofurantoin and 34.38% resistance to ciprofloxacin (Table 2). Similarly, Table 3 shows the MARI index of the isolated uropathogens to the antibiotics tested. On average, the tested isolates had MARI greater than 0.2, implying that the UPEC strains are resistant to at least two or more antibiotics from different classes, thus connoting to the multidrug resistance nature of the *E. coli* isolates investigated in this study.

Phenotypic and molecular screening of the uropathogenic *E. coli* for ESBL. Table 4 shows the results of the phenotypic and molecular detection of ESBL production in the test *E. coli* isolates investi-

Table 1. Primer sequence of the ESBL Genes.

Genes	Oligonucleotide sequence	Amplicon size (bp)	Temperature (°C)
TEM F	AGTGCTGCCATAACCATGAGTG	431	60
TEM R	CTGACTCCCCGTCGTGTAGATA	431	60
SHV F	GATGAACGCTTTCCCATGATG	214	60
SHV R	CGCTGTTATCGCTCATGGTAA	214	60
CTX F	ATGTGCAGYACCAGTAARGT	593	50
CTX R	TGGGTRAARTARGTSACCAGA	593	60
CTX-M-15 F	CACGTCAATGGGACGATGT	410	60
CTX-M-15 R	GAAAGGCAATACCACCGGT	410	60

Key: F – forward primers, R- reverse primers

Table 2. The antimicrobial susceptibility pattern of the isolates to the selected antibiotics.

Antibiotics (µl)	Resistance (%)	Susceptible (%)
Ceftazidime (30)	76.88	23.12
Cefuroxime (30)	77.5	22.5
Gentamicin (30)	6.25	93.75
Cefixime (30)	61.88	38.12
Ofloxacin (10)	6.88	93.12
Augmentin (30)	32.5	67.5
Nitrofurantoin (10)	0.63	99.37
Ciprofloxacin (10)	34.38	65.62

Table 3. MARI of uropathogenic *E. coli* isolates

MARI Index	Number of Isolates
0.1	19
0.2	29
0.3	66
0.5	39
0.7	06
0.8	01

Table 4. Frequency of ESBL positive *E. coli* isolates

Test	n (%)
Combined disk method	
ESBL negative	116 (72.5)
ESBL positive	44 (27.5)
PCR	
<i>bla_{TEM}</i> gene	8 (5.0)
<i>bla_{SHV}</i> gene	7 (4.4)
<i>bla_{CTX-M-15}</i> gene	11 (6.9)

gated in this study using the combined disk technique and PCR techniques respectively. A total of 8 isolates of *E. coli* (5.0%) were confirmed by PCR to harbour the *bla_{TEM}* ESBL gene – that mediates resistance to the cephalosporins. The *bla_{SHV}* gene mediates bacterial resistance to the β-lactams, and this gene was detected in a total of 7 *E. coli* isolates (4.4%) tested for ESBL gene by PCR. The most predominant ESBL gene confirmed by PCR in this study was the *bla_{CTX-M-15}* ESBL gene – which is unique in mediating bacterial resistance to cefotaxime, an important third generation cephalosporin that has wide applications in clinical medicine for the treatment of a wide variety of infections caused by pathogenic Gram-negative bacteria. A total of 11 *E. coli* isolates (6.9%) were confirmed to harbour the *bla_{CTX-M-15}* ESBL gene (Table 4). Table 5 shows the resistance profile of the ESBL-producing *E. coli* isolates to some commonly used antibiotics in the hospital environments.

DISCUSSION

Urinary tract infections (UTIs) are among the commonest human bacterial infections occurring both in the community and hospital settings particularly in developing countries with a high rate of casualty (3).

Table 5. Resistance profile of ESBL-UPEC strains harbouring *bla_{TEM}* and *bla_{CTX-M-15}* genes to β-lactam antibiotics

Antibiotics	<i>bla_{TEM}</i> (%)	<i>bla_{CTX-M-15}</i> (%)
Cefuroxime	100	100
Cefixime	77.8	0
Amoxicillin-clavulanic acid	77.8	100
Ceftazidime	100	75

The prevalence of uropathogenic *E. coli* (UPEC) in our study was 44.1%. This is higher than the prevalence rate of 5.8 % recorded in Jos, North Central Nigeria (16). However, a higher prevalence of 58.2% was recorded among HIV-positive patients in Poland by Skrzat-Klapaczyńska et al. (17). It is surprising that despite co-trimoxazole use as a prophylaxis for

the prevention of opportunistic infections among HIV positive individuals in Awka Metropolis, we still have a high prevalence of UTI among the patients. UTI incidence in HIV positive patients have been related to infection and immune functions determined by lymphocytes CD4⁺ cells count. The incidence increases with decreasing immune function (17). The antibacterial activities of the antibiotics tested against the UPEC isolates in this study were in the order: cefuroxime < ceftazidime < cefixime < ciprofloxacin < augmentin < ofloxacin < gentamicin < nitrofurantoin. This shows that nitrofurantoin, gentamicin and ofloxacin were highly active and thus can be used for the treatment of UTIs caused by uropathogenic strains of *E. coli* in HIV patients. This finding agrees with the findings of Ekwealor et al. (2) who also showed in their study that nitrofurantoin is the most active antibacterial agent in cases of UTI. In another related study, Kanu et al. (18) recorded a similar finding, stating that gentamicin and ciprofloxacin were the drugs of choice for the treatment of UTI in people living with HIV. The high degree of resistance of these UPEC strains from HIV patients might be because HIV patients are immune-compromised and thus have been exposed to a lot of infections and in turn, lots of antibiotics are used in their management. Antibiotic misuse and abuse have been recorded as major ways of promoting antibiotic resistance in a population of animals or humans (2, 19). Strikingly, the majority of the UPEC isolates from our study showed resistance to the cephalosporins. Emergence of β -lactamases producers among the uropathogenic *E. coli* reduces therapeutic options because the isolates often co-express resistance to other classes of antibiotics (3, 20). A 61.36% of the cephalosporins resistant isolates in our study were phenotypically and genotypically confirmed to be ESBL producers. Zenati et al. (21) reported a similar prevalence rate of ESBL producing *E. coli* (32.5%). On the molecular level, among the three (3) ESBL resistance genes checked, the predominant gene was $bla_{CTXM-15}$ (6.9%) genes, followed by bla_{TEM} (5.0%). The isolates that harbored the bla_{TEM} resistant gene were

predominantly resistant to cefuroxime (100%), cefixime (77.8%), amoxicillin-clavulanic acid (77.8%) and ceftazidime (100%) – which are all broad-spectrum and last-line antibiotics used to manage serious bacterial infections including those caused by pathogenic strains of *E. coli*. Similarly, the isolates that harbored the $bla_{CTXM-15}$ resistant gene were found

to be predominantly resistant to cefuroxime (100%), amoxicillin-clavulanic acid (100%) and ceftazidime (75%). Our findings differed from that of a similar work done by Padmavathy et al. (8) in India. They reported that 70.2% of the ESBL positive isolates have the bla_{CTXM-M} gene. They also reported that these isolates were found to be more resistant to ciprofloxacin (92.5%) and gentamicin (75%), which totally does not agree with our findings. Extended-spectrum β -lactamases are frequently identified in *E. coli* and are responsible for resistance against penicillins, cephalosporins, aztreonam and other β -lactam and some non- β -lactam agents (22-23). In this study, we report significant resistant patterns (above 50%) of the uropathogenic *E. coli* (UPEC) isolates from HIV patients when compared to previous studies in this region in which the antimicrobial susceptibility patterns of UPEC strains was in the range of 20-40% (1-2). Following an extensive use of β -lactam antibiotics over the years, various β -lactamases types and mutants have emerged both in hospital and community settings. The CTX-M enzymes have been reported to cause an increase in the treatment failure of both complicated and uncomplicated UTIs (21). The levels of ESBL producers are a major threat to infection management as this may have contributed to the antibiotic resistance reported in this study. ESBL-producing organisms are known to contain plasmids with genes that encode resistance to other antibiotics (3). It has been severally reported that ESBL-producing *E. coli* isolates exhibit an alarming trend in co-resistance to other classes of antibiotics (8). In our findings, the PCR technique had better detection capacity than the phenotypic method because it looked for the genes responsible for ESBL production, unlike the phenotypic tests which is more of a general tests that detect both ESBL and other similar AMR mediators. It has been previously reported that occurrence of many ESBL types or ESBL-AmpC combinations within the same organism make phenotypic screening of the β -lactamases difficult and non-reliable (3, 24).

The preliminary epidemiological data from our

study gives credence to these reported claims, and therefore reiterates the need for the proper surveillance, detection and reporting of multidrug resistance cases in our hospital's laboratories to guide therapy and thus ensure good prognosis of affected patients. Such measures will help to stop the emergence and spread of resistant bacteria in both the community and hospital environment.

CONCLUSION

This study reported a 44.1% prevalence of uropathogenic *E. coli* among HIV/AIDS patients in Awka Metropolis, Nigeria. Our study shows the occurrence of ESBL genes in uropathogenic multidrug-resistant UPEC strains among HIV patients in Awka metropolis, Nigeria. The predominant gene detected was *bla*_{CTXM-15} (6.9%). It is our hope that findings of this magnitude will help clinicians to better define the empiric treatment of UTIs caused by multidrug-resistant *E. coli* especially among HIV positive individuals in Nigeria.

REFERENCES

1. Ejikeugwu C, Ikegbunam M, Ugwu C, Eze P, Iroha I, Charles E. Phenotypic detection of *Klebsiella pneumoniae* strains-producing Extended Spectrum β -Lactamase (ESBL) enzymes. *Sch Acad J Biosci* 2013; 1: 20-23.
2. Ekwealor PA, Ugwu MC, Ezeobi I, Amalukwe G, Ugwu BC, Okezie U, et al. Antimicrobial evaluation of bacterial isolates from urine specimen of patients with complaints of urinary tract infections in Awka, Nigeria. *Int J Microbiol* 2016; 2016: 9740273.
3. Ugwu MC, Shariff M, Nnajide CM, Beri K, Okezie UM, Iroha IR, Esimone CO. Phenotypic and molecular characterization of β -Lactamases among Enterobacterial Uropathogens in South-Eastern Nigeria. *Can J Infect Dis Med Microbiol* 2020; 2020: 5843904.
4. Iregbu KC, Nwajiobi-Princewill PI. Urinary tract infections in a tertiary hospital in Abuja, Nigeria. *Afr J Clin Exp Microbiol* 2013; 14: 169-173.
5. Abujnah AA, Zorgani A, Sabri MA, El-Mohammady H, Khalek RA, Ghenghesh KS. Multidrug resistance and extended-spectrum β -lactamases genes among *Escherichia coli* from patients with urinary tract infections in Northwestern Libya. *Libyan J Med* 2015; 10: 26412.
6. Elsayed TI, Ismail HA, Elgamel SA, Gad AH. The Occurrence of multidrug resistant *E. coli* which produce ESBL and cause urinary tract infections. *J Appl Microbiol Biochem* 2017; 1: 1-8.
7. Xavier TF, Auxilia A, Auxilia A, Kannan M. Isolation and characterization of UTI pathogens from HIV positive patients of Karur district, Tamil Nadu, India. *Int J Curr Microbiol Appl Sci* 2015; 4: 558-563.
8. Padmavathy K, Padma K, Rajasekaran S. Multidrug resistant CTX-M-producing *Escherichia coli*: A growing threat among HIV patients in India. *J Pathog* 2016; 2016: 4152704.
9. Jadhav S, Hussain A, Devi S, Kumar A, Parveen S, Gandham N, et al. Virulence characteristics and genetic affinities of multiple drug resistant uropathogenic *Escherichia coli* from a semi urban locality in India. *PLoS One* 2011; 6(3): e18063.
10. Waheed A, Saleem S, Shahzad N, Akhtar J, Saeed M, Jameel I, et al. Prevalence of extended spectrum β -lactamase SHV and OXA producing gram negative bacteria at tertiary care hospital of Lahore, Pakistan. *Pakistan J Zool* 2019; 51: 2345-2351
11. Malande OO, Nuttall J, Pillay V, Bamford C, Eley B. A ten-year review of ESBL and non-ESBL *Escherichia coli* bloodstream infections among children at a tertiary referral hospital in South Africa. *PLoS One* 2019; 14(9): e0222675.
12. Cheesbrough M (2004). District laboratory practice in tropical countries. 2nd ed. Cambridge University Press. UK.
13. Wayne PA (2017). Clinical and Laboratory Standards Institute (CLSI). Performance standards for Antimicrobial susceptibility testing. 17th ed. CLSI supplement M100.
14. Ejikeugwu PC, Ugwu CM, Araka CO, Gugu T, Iroha I, Adikwu M, et al. Imipenem and meropenem resistance amongst ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates. *Int Res J Microbiol* 2012; 3: 339-344.
15. Ejikeugwu C, Iroha I, Amaechi CO, Ugwu M, Eze P, Iroha CS, et al. Multiple antibiotic resistance, antibiogram and phenotypic detection of Metallo-Beta-Lactamase (MBL) from *Escherichia coli* of poultry origin. *J Appl Microbiol Biochem* 2017; 1: 1-5.
16. Zakka S, Oluwatosin OC, Cosmas EC, John S, Rebecca EF, Isa BE. Prevalence of urinary tract infection in HIV patients on antiretroviral drugs in Jos Metropolis, Nigeria. *World J Pub Health* 2018; 3: 57-60.
17. Skrzat-Klapaczyńska A, Matłoz B, Bednarska A, Paciorek M, Firląg-Burkacka E, Horban A. Factors associated with urinary tract infections among HIV-1 infected patients. *PLoS One* 2018; 13(1): e0190564.
18. Kanu AM, Mgbajiaka N, Abadom N. Prevalence of urinary tract infection among HIV patients in Abuja, Nigeria.

- ria. *Int J Infect Dis* 2016; 45: S1-229.
19. Ugwu MC, Ikegbunam MN, Nduka SO, Attama AA, Ibezim EC, and Esimone CO. Molecular characterization and efficacy of antibiotic combinations on multiple antibiotic resistant *Staphylococcus aureus* isolated from nostrils of healthy human volunteers. *J Pharm Sci Res* 2013; 5: 26-32.
 20. Ejikeugwu C, Ugwu M, Iroha I, Gugu T, Duru C, Eze P, et al. Detection and antimicrobial susceptibility of some Gram-negative bacteria producing carbapenemases and extended spectrum β -Lactamases. *Int J Microbiol Immunol Res* 2013; 2: 064-069.
 21. Zenati F, Barguigua A, Nayme K, Benbelaid F, Khadir A, Bellahsene C, et al. Characterization of uropathogenic ESBL-producing *Escherichia coli* isolated from hospitalized patients in western Algeria. *J Infect Dev Ctries* 2019; 13: 291-302.
 22. Bali EB, Acik L, Sultan N. Phenotypic and molecular characterization of SHV, TEM, CTX-M and extended-spectrum beta-lactamase produced by *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella* isolates in a Turkish hospital. *Afr J Microbiol Res* 2010; 4: 650-654.
 23. Bailey JK, Pinyon JL, Anantham S, Hall RM. Commensal *Escherichia coli* of healthy humans: A reservoir for antibiotic-resistance determinants. *J Med Microbiol* 2010; 59: 1331-1339.
 24. Padmavathy K, Padma K, Rajasekaran S. Extended spectrum β - lactamase/AmpC-producing uropathogenic *Escherichia coli* from HIV patients: do they have a low virulence score? *J Med Microbiol* 2013; 62: 345-351.