Prevalence and multidrug-resistant ESBL-producing E. coli in urinary tract infection cases of HIV patients attending Federal Teaching Hospital, Abakaliki, Nigeria

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The study determined the frequency of extended-spectrum beta-lactamase-producing Escherichia coli (ESBL-PEC) in HIV-infected individuals with urinary tract infections (UTIs) attending Federal Teaching Hospital Abakaliki (FETHA II), and the responses of these bacterial pathogens to colistin, cephalosporins, aminoglycosides, fluoroquinolones, and ertapenem. Exactly 200 urine samples (mid-stream) were collected from HIV-infected individuals. Standard microbiological techniques were used to characterize bacterial isolates. Phenotypic screening for the production of ESBLs was done by double disc synergy test. Antibiotic susceptibility study was carried out by the Kirby-Bauer disc diffusion technique. Results showed the presence of ESBL-PEC in the urine samples of HIV-infected individuals. ESBL-PEC were highly resistant to gentamycin (85%), ofloxacin (75%), ciprofloxacin (75%), nalidixic acid (70%), tobramycin (65%), kanamycin (64.3%), and norfloxacin (60%), but susceptible to ertapenem (60%) and amikacin (57.1%). The ESBL-PEC isolates were multidrug-resistant. Average multiple antibiotic resistance indices (MARI) value of isolates was 0.8, further depicting misuse/abuse of these antibiotic classes in our study area. Thus, it is pertinent to carry out antibiotic susceptibility testing before the commencement of antibiotic therapy, especially in HIV-positive patients with UTIs so as to attain effective treatment regimens and reduce the incidence of antibiotic resistance in healthcare settings.

Key words: Escherichia coli, extended-spectrum beta-lactamase (ESBL), multidrug resistance, antibiotics, urinary tract infections (UTI), human immunodeficiency virus (HIV) patients.

INTRODUCTION

Urinary tract infection (UTI) is an infection occurring in any region of the urinary system such as ureters, bladder, kidneys, and urethra. UTI caused by pathogenic bacteria, especially ESBL-producing microorganisms has resulted in life-threatening sepsis (Tessema et al., 2020). Treatment of uncomplicated UTIs may be with
fluoroquinolones if the causative organism is sensitive to them, but increase in the resistant bacteria causing UTIs has caused limitations in their action in today’s health sector (Marwa et al., 2015). Human immunodeficiency virus (HIV)-positive patients are liable to have opportunistic infections like UTIs, especially among people living in developing countries where proper healthcare facilities are limited (Foxman, 2002; Abongomera et al., 2021). Studies have shown high frequency (41 - 52%) of asymptomatic bacteriuria in HIV patients (Awolade et al., 2010; Ezechi et al., 2013), and the routine administration of trimethoprim-sulfamethoxazole prophylaxis among HIV-positive patients might increase the prevalence of multidrug-resistant (MDR) bacterial infections (Ezechi et al., 2013; Olaru et al., 2021). Extended-spectrum beta-lactamase (ESBLs) are a class of plasmid-mediated diverse, complex and rapidly evolving enzymes which can hydrolyze penicillins, monobactams, and broad-spectrum cephalosporins. Plasmids carrying gene-coding ESBLs often bear genes encoding resistance to various antibiotic classes; for instance, aminoglycosides and fluoroquinolones. Hence, getting antimicrobial agents against ESBL-producing microorganisms in healthcare is often difficult (Ruppe et al., 2015). Extended-spectrum beta-lactamase-producing Escherichia coli (ESBL-Ec) have been reported in non-hospitalized and hospitalized patients (Marwa et al., 2015). The spread of ESBLs and other forms of resistance to antibiotics is an international threat to the health sector. The misuse of antibiotics is one of the major factors causing an increase in resistance of antibiotics by bacteria in Abakaliki metropolis (Ozlem et al., 2007; Moses et al., 2018, 2020). Therefore, this study is designed to evaluate the frequency of ESBL-Ec isolated from urine samples of HIV-positive patients with UTIs attending Federal Teaching Hospital Abakaliki (FETHA), and the responses of these bacterial pathogens to selected members of four commonly used antibiotic classes (cephalosporins, aminoglycosides, fluoroquinolones, and carbapenem).

MATERIALS AND METHODS

Clinical samples collection

Two hundred mid-stream urine samples (129 from females and 71 from males) of HIV-positive patients attending FETHA II were used for this study between February and November, 2019. Urine samples were collected from both the medical ward and outpatient department of the hospital as clean catch urine with sterile 30 ml polystyrene universal containers. Collected samples were labeled appropriately and immediately transported in ice packs to the laboratory for bacteriological analysis within 30 and 60 min.

Ethical clearance

Ethical clearance for the study was approved by ethical clearance committee of FETHA (FETHA/REC/VOL 2/2019/233), Ebonyi State, Nigeria. Informed consent was also obtained from each patient before sample collection.

Isolation and phenotypic characterization of bacterial isolates from urine samples

Collected mid-stream urine samples were aseptically streaked on MacConkey agar. They were then incubated for 18 h at 37°C. At the completion of incubation, plates were observed for E. coli growth (red or pink colonies) on MacConkey agar, sub-cultured on eosin methylene blue (EMB) agar, and incubated for 24 h at 37°C. EMB agar plates were thereafter observed for E. coli growth (green metallic sheen appearance) after incubation. These suspected bacterial isolates were then characterized with standard microbiological methods: Gram-staining, motility, sugar fermentation, and other biochemical tests such as indole, methyl red, Voges-Proskauer, citrate, motility, and urease test. The pure isolates were further inoculated on nutrient agar slants, incubated for 24 h at 37°C, and stored in the refrigerator at 4°C for future use (Cheesebrough, 2010; Moses et al., 2018).

Detection of ESBL by double-disc synergy test (DDST)

The bacterial isolates that exhibited reduced susceptibility to any of the 2nd and 3rd generation cephalosporins were phenotypically confirmed for ESBL production using the double-disc synergy test (Iroha et al., 2010). DDST was performed as a standard disc diffusion assay on Mueller-Hinton (MH) agar (Oxoid, UK) plates in line with CLSI criteria (CLSI, 2015). The plates were incubated at 37°C for 18 to 24 h. ESBL production was suspected phenotypically when the zones of inhibition of the cephalosporins (cefotaxime 30 µg and ceftazidime 30 µg) increased in the presence of amoxicillin/clavulanic acid disk (20/10 µg). Az 5 mm increase in the inhibition zone diameter for either of the cephalosporins (ceftaxime and ceftazidime) tested in combination with amoxycillin-clavulanic acid versus its zone when tested alone confirmed ESBL production phenotypically (CLSI, 2015; Iroha et al., 2010).

Antibiotic susceptibility test

Antibiotic susceptibility of the E. coli isolates was determined using the Kirby-Bauer disc diffusion method according to the recommendations of the Clinical and Laboratory Standard Institute CLSI (CLSI, 2016). The isolates were sub-cultured on nutrient agar and incubated at 37°C for 24 h. Then the colonies of each of the isolate were adjusted to 0.5 McFarland turbidity standard (equivalent to 1.5×10⁶ cfu/ml), incubated for 10 min and inoculated onto Mueller-Hinton agar plates using sterile swab sticks. The surface of the medium was streaked in four directions while the plates were rotated approximately 60° to ensure even distribution. The inoculated Mueller-Hinton agar plates were allowed to dry for a few minutes. Standard antibiotic discs tested against the isolates were ceftazidime (CAZ) (30 µg), amikacin (AK) (30 µg), cefotaxime (CTX) (30 µg), tobramycin (Tob) (10 µg), gentamicin (GC) (10 µg), kanamycin (K) (5 µg), ciprofloxacin (CIP) (5 µg), ofloxacin (O FX) (5 µg), norfloxacin (NOR) (10 µg), cefotetan (CTT) (30 µg),

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The inhibitory zone diameter was interpreted as susceptible, intermediate or resistant according to the criteria of CLSI (CLSI, 2018). Comparison of frequencies to which the isolates were ESBL producers out of the total number of antibiotics tested against the isolates (b) according to Moses et al. (2020). The average MARI value of the ESBL-PEc isolates was 0.8.

**RESULTS**

Results showed that 37 (37%) isolates were ESBL producers out of 100 E. coli isolates obtained from females, while 21 (42%) ESBL-PEc were obtained from the 50 E. coli isolates from males (Table 1). There was no statistically significant difference in the prevalence frequencies of E. coli in urine samples of male and female HIV-positive patients from FETHA II (P < 0.05). There was also no statistically significant difference in the prevalence frequencies of ESBL-producing E. coli in urine samples of male and female HIV-positive patients from FETHA II (P < 0.05).

Antibiotic susceptibility test results of ESBL-PEc isolates against cephalosporins and carbapenem (ertapenem) revealed that isolates were very resistant to cefepime (78%), aztreonam (73%), ceftazidime (70%), cepotetan (65%), and cefotaxime (58%), but susceptible to ceftriaxone (52%) and ertapenem (60%). All the isolates were completely susceptible to cefuroxime (100%) (Table 2). Antibiotic susceptibility test results of ESBL-PEc isolates against aminoglycosides and fluoroquinolones also revealed that isolates exhibited high resistance to gentamycin (85%), ofloxacin (75%), ciprofloxacin (75%), nalidixic acid (70%), tobramycin (65%), kanamycin (64.3%), and norfloxacin (60%) but susceptible to amikacin (42.9%) (Table 3).

The DISCUSSION

Bacterial infections increase the rate of mortality and morbidity among HIV-infected patients because of cell-mediated and humoral immunity defects (Moremi et al., 2014). Many researchers have revealed that there is a relationship between HIV and opportunistic infections (Ya et al., 2008; Spach and Jackson, 2010). ESBL-producing E. coli (ESBL-PEc) usually associated with UTIs in HIV-

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**Table 1. Frequency of ESBL-producing E. coli isolates in urine samples of HIV-positive patients in FETHA II.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Female No. of samples screened</th>
<th>Female No. of E. coli isolated n (%)</th>
<th>Female No. of ESBL-producers</th>
<th>Male No. of samples screened</th>
<th>Male No. of E. coli isolated n (%)</th>
<th>Male No. of ESBL-producers</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤18</td>
<td>26</td>
<td>18 (69.2)</td>
<td>3</td>
<td>11</td>
<td>3 (27.3)</td>
<td>1</td>
</tr>
<tr>
<td>19 - 29</td>
<td>14</td>
<td>13 (92.9)</td>
<td>8</td>
<td>9</td>
<td>6 (66.7)</td>
<td>6</td>
</tr>
<tr>
<td>30 - 39</td>
<td>56</td>
<td>46 (82.1)</td>
<td>11</td>
<td>28</td>
<td>23 (82.1)</td>
<td>8</td>
</tr>
<tr>
<td>≥ 40</td>
<td>33</td>
<td>23 (69.7)</td>
<td>15</td>
<td>23</td>
<td>18 (78.3)</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>100 (77.5)</td>
<td>37 (37%)</td>
<td>71</td>
<td>50 (70.4)</td>
<td>21 (42%)</td>
</tr>
</tbody>
</table>

**Table 2. Percentage susceptibility frequency of ESBL-producing E. coli isolated from urine samples of HIV-positive patients to cephalosporins and ertapenem in FETHA II.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antibiotics</th>
<th>CAZ</th>
<th>CTX</th>
<th>CTR</th>
<th>FEP</th>
<th>CRO</th>
<th>ATM</th>
<th>CXM</th>
<th>ETP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance (%)</td>
<td>70</td>
<td>58</td>
<td>65</td>
<td>78</td>
<td>48</td>
<td>73</td>
<td>0</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Susceptible (%)</td>
<td>30</td>
<td>42</td>
<td>35</td>
<td>22</td>
<td>52</td>
<td>27</td>
<td>100</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

CTX = Cefotaxime, ATM = Aztreonam, CTT = Cefotetan, CRO = Ceftriaxone, CXM = Cefuroxime, CAZ = Ceftazidime, ETP = Ertapenem, FEP = Cefepime.

**Multiple antibiotic resistance index determination**

Multiple antibiotic resistance index (MARI) of the isolates was calculated as the number of antibiotics to which the isolates exhibited resistance (a), divided by the total number of antibiotics tested against the isolates (b) according to Moses et al. (2020).

MARI = a/b.

**Statistical analysis**

Statistical analysis was performed using SPSS version 17.0 statistical software package (Moses et al., 2018). Comparison between categorical variables was calculated using Independent Samples T-test. Results were considered statistically significant if the p value was less than 0.05 (p < 0.05).
positive patients are growing in prevalence and causing serious burden in the health sector (Kemajou et al., 2016).

The present study was designed to determine the frequency of ESBL-producing E. coli in HIV-infected individuals attending Federal Teaching Hospital Abakaliki (FETHA II) with UTIs and the responses of these bacterial pathogens to cephalosporins, aminoglycosides, fluoroquinolones, and ertapenem. One hundred and fifty [150 (75%)] E. coli isolates (100 from females and 50 from males) were obtained from 200 mid-stream urine samples of HIV-positive patients. Interestingly, this study showed that cefuroxime was the most effective antibiotic against the E. coli isolates as all the isolates were completely susceptible (100%). Ertapenem and ceftriaxone were effective against ESBL-PEc isolates with susceptibility frequencies of 60 and 52%, respectively.

ESBL-PEc isolates in our study were also generally highly resistant to aminoglycoside and fluoroquinolones. However, amikacin was the most effective aminoglycoside antibiotic against the isolates as 57.1% of the isolates were susceptible. All ESBL-PEc isolates in this study were multidrug-resistant as they were resistant to at least three different antibiotic classes. Their multidrug resistance tendencies were further depicted by their average MARI value of 0.8. The frequency of E. coli in the urine samples of HIV-positive patients with UTIs (77.5% from females and 70.4% from males) is in concord with the work of Kemajou et al. (2016) who reported 141 (57.3%) E. coli isolates from 286 urine samples of HIV-positive patients (74.4% were from females while 25.6% were from males). The differences in the frequency of E. coli in the urine samples could be as a result of the larger number of urine samples from females (129) than males (71). The results of this study also agree with that conducted in Iran by Serkadis et al. (2014) who reported 19.3% ESBL-producing E. coli isolates from 250 urine samples of HIV-positive outpatients screened even though we recorded higher frequency (42% from males and 37% from females) values in our study. They also reported that E. coli was the most common uropathogenic bacteria in 114 (59.1%) of UTIs.

This study did not completely agree with the study of Inyang Etoh et al. (2009) in Calabar and Omorogie and Eghefona (2009) in Benin. Nigeria who reported a higher frequency of ESBL-producing E. coli in urine samples of females than males with UTIs. The outcome of higher UTI in females may be because of the predominance of E. coli in different populations and the proximity between vagina and anus, complex physiology during pregnancy, intake of contraceptives, and urethra in relation to personal hygiene (Thakur et al., 2013). Even though more urine samples were collected from females (129) than males (71), the high frequency of ESBL-producing E. coli (ESBL-PEc) reported among males (42%) in our study could be as a result of the high number of positive males with UTIs. Our study reported that the age between 19-39 years (82%) had the highest frequency of E. coli associated with UTI while the least E. coli frequency was in the age group < 18 (56%). This is in agreement with previous work (Thakur et al., 2013; Bomjan, 2005; Livermore and Hawkey, 2005; Prakash et al., 2009), where high E. coli frequencies were reported in urine between the age groups of 20 to 40 years. This could be due to the established report that the incidence of UTIs increases with sexual activity and age. However, our study disagreed with the work of Thakur et al. (2013) who previously reported high incidence of UTIs in old age males. This high incidence of E. coli in the urine samples of older men could be as a result of some circumstances such as diabetes, prostatitis, prolonged treatment with antibiotics, and weakened immune system (Thakur et al., 2013).

The isolates in this present study were generally resistant to cephalosporins, aminoglycosides, and fluoroquinolones. This is in agreement with the work of Perez et al. (2007) who indicated that 94% of E. coli isolates exhibited resistance to cephalosporins. This high resistance frequency might be as a result of ineffective antibiotic policy and the irrational use of cephalosporins in hospitals (Gonzalez-pedilla et al., 2015). High rate of E. coli resistance to cephalosporins is mainly due to the production of ESBLs; this was recorded from several countries (Bouchillon et al., 2004; Khanfar et al., 2009). However, self-medication practice, the use of antibiotics without doctor’s prescription, easy availability of drugs from the pharmacy, and loop holes in guidelines in antimicrobial usage policies in developing countries such

Table 3. Percentage susceptibility frequency of ESBL-producing E. coli isolated from urine samples of HIV-positive patients to aminoglycosides and fluoroquinolones in FETHA II.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aminoglycosides</th>
<th>Fluoroquinolones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOB</td>
<td>K</td>
</tr>
<tr>
<td>Resistance (%)</td>
<td>65</td>
<td>64.3</td>
</tr>
<tr>
<td>Susceptible (%)</td>
<td>35</td>
<td>35.7</td>
</tr>
</tbody>
</table>

TOB = Tobramycin, K = Kanamycin, AK = Amikacin, CN = Gentamicin, OFX = Ofloxacin, CIP = Ciprofloxacin, NA = Nalidixic acid, NOR = Norfloxacin.
as Nigeria may be a major contributing factor to antibiotic resistance (Moses et al., 2018).

Interestingly, isolates in this study were susceptible to cefuroxime (100%), ceftriaxone (52%), and ertapenem (60%); while they exhibited high resistance to cefepime (78%), aztreonam (73%), ceftazidime (70%), cefotetan (65%), and cefotaxime (58%). Our study partially agrees with the work of Serkadis et al. (2014) in Iran who reported that E. coli isolates from 87 HIV-positive outpatients were highly susceptible to nitrofurantoin, ceftazidime, cefoxitin, ceftriaxone, aztreonam, cefotaxime, and ciprofloxacin with resistance frequencies of 95.4, 94.3, 93.1, 91.95, 90.8, 89.6 and 72.4%, respectively. Our study showed that 37 (37%) isolates were ESBL producers out of the 100 E. coli isolates obtained from females, while 21 (42%) ESBL-P Ec were obtained from the 50 isolates from males. This agrees with the study of Osazuwa et al. (2011) who reported ESBL-producing E. coli isolates in urine samples of HIV-infected. This study also concurs with the work of Iroha et al. (2017) who reported ESBL-producing E. coli in urine samples of HIV-positive individuals in Abakaliki, Nigeria.

Fluoroquinolones are widely used in the empirical therapy of UTI. They are the drug of choice for the treatment of infections due to ESBL-producing microbes (Pitout and Laupland, 2008). Nevertheless, a previous study reported a strong association between ESBL production and fluoroquinolone resistance (Frank et al., 2011). In our study, the resistance exhibited by ESBL-producing E. coli isolates to fluoroquinolones (ofloxacin, ciprofloxacin, norfloxacin, and nalidixic acid) and aminoglycosides (tobramycin, kanamycin, gentamicin, except amikacin) agrees with other studies that reported an increased resistance towards fluoroquinolones, aminoglycosides, and folate pathway inhibitors (Pitout and Laupland, 2008; Prakash et al., 2009). The average MARI value of the ESBL-producing E. coli isolates in this study was 0.8. Multiple antibiotic resistance index (MARI) of uropathogens is increasing worldwide. MARI usually varies according to geographic locations, but it is usually directly proportional to the misuse of antibiotics (Iroha et al., 2017).

Conclusion

This study has shown that E. coli is a causative agent of UTI in HIV-positive patients attending FETHA II. Results showed that E. coli isolates implicated in UTIs among HIV-positive patients are multidrug-resistant ESBL producers with co-resistances to aminoglycosides and fluoroquinolones. Cefuroxime, ceftriaxone, ertapenem, and amikacin were the most active antibiotics against ESBL-P Ecol isolates. The increasing prevalence of ESBL-P Ecol among HIV-positive patients with UTIs poses serious challenges in healthcare settings. Therefore, it is imperative to carry out antibiotic susceptibility testing before the commencement of antibiotics therapy in HIV-positive patients with UTIs so as to ascertain the most current effective antibiotics. This will greatly help in achieving appropriate antibiotic therapy and curbing the increasing antibiotics resistance menace.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

REFERENCES


