Original Research Article

Detection of ESBL and AmpC β-lactamases producing in uropathogen Escherichia coli isolates at Benghazi Center of Infectious Diseases and Immunity

Muna Mohammed Buzayan1* and Fauzia Rajab El-Garbulli2

1Benghazi Center of Infectious Diseases and Immunity (BCIDI), Benghazi, Libya
2Department of Botany, Faculty of Science, Benghazi University, Benghazi, Libya
*Corresponding author

ABSTRACT

Multidrug resistance in uropathogens is increasing worldwide in both hospitalized and unhospitalized patients. Antibiotic-resistant uropathogen spread makes empirical treatment of Urinary Tract Infections (UTIs) difficult. The aim of the current study is to determine the prevalence of antimicrobial resistance to antibiotics currently used to treat Urinary Tract Infections (UTIs), extended spectrum β-lactamases (ESBLs) production and AmpC β-lactamases production among Escherichia coli isolated from UTI Human Immunodeficiency Virus (HIV) positive and negative out-patients in Benghazi Center of Infectious Disease and Immunity (BCIDI). A total of 114 E. coli isolates were collected from UTI HIV-positive and HIV-negative out-patients from January 2011 to December 2012. The antimicrobial susceptibility to various drugs was studied by the disc diffusion method as guided by the Clinical and Laboratory Standards Institute (CLSI) guidelines. Confirmation of the ESBLs production and AmpC β-lactamases production was done by using Double- disk synergy test (DDST) and Modified three-dimensional test, respectively. In the present study, the highest antibiotics resistance in the E. coli strains from HIV positive and HIV negative out-patients was to ampicillin, nalidixic acid and trimethoprim-sulfamethoxazole (77.8% vs. 55.17%), (59.3% vs. 39.08%) and (55.6% vs. 36.8%). Prevalence of ESBL and AmpC β-lactamase and the coexistence of the phenotype (ESBL+ AmpC β-lactamase) in E. coli in positive and negative HIV out-patients were found to be 48.14% vs 12.64%, 0% vs 0% and 3.7% vs 1.15%, respectively. The ESBL producers are found in HIV positive out-patients more than in HIV negative out-patients. Nitrofurantoin was found to be the most effective antimicrobials. Our data analysis recommends nitrofuranto in as empirical therapy against E. coli causing UTI.

Introduction

Multidrug resistance in uropathogens is increasing worldwide in both hospitalized and unhospitalized patients. It varies according to geographic locales but it is usually directly proportional to the use and misuse of antibiotics (Gales et al., 2000).
Klebsiella spp. and Escherichia coli are the most common produce Extended-spectrum beta-lactamases (ESBLs) and AmpC β-lactamases but may also occur in other gram-negative bacteria. Both ESBLs and AmpC β-lactamases are associated with broad multidrug resistance (Thomson, 2001). ESBL-producing bacteria are frequently resistant to many of antibiotics, making it difficult to treat infections (Nathisuwan et al., 2001). ESBLs cause bacterial resistance to the penicillins, first-, second-, and third - generation cephalosporins, and aztreonam (but not the cephemycins or carbapenems) by hydrolysis of these antibiotics and are inhibited by clavulanic acid (Paterson & Bonomo, 2005). AmpC β-lactamases are clinically important because they confer resistance to α-methoxy-β-lactams, such as cefoxitin, narrow-, expanded-, and broad-spectrum cephalosporins, aztreonam and are not inhibited by clavulanic acid or other β-lactamase inhibitors (Coudron et al., 2000).

Bacterial infections of Human Immunodeficiency Virus (HIV) patients increase the rate morbidity and mortality among those patients because of the defects in both the cell-mediated and humoral immunity (Adeleye et al., 2008). The management of bacterial infection in patients infected with HIV is important because the HIV disrupts the body’s own disease fighting immune system (Waikhom & Devi, 2012). Detection of ESBL production and AmpC β-lactamases in organisms from urine samples may be important because this represents an epidemiologic marker of colonization and play an important role in the selection of appropriate therapy (Harris et al., 2007).

The aim of current study is to determine the prevalence of antimicrobial resistance to antibiotics currently used to treat UTIs, ESBL production and AmpC β-lactamases production among E. coli isolated from UTI-patients with HIV-positive and negative out-patients in Benghazi Center of Infectious Disease and Immunity (BCIDI).

Materials and Methods

Bacterial isolates

A total of 1000 clean-catch (midstream) urine samples were processed during two years study (from January 2011 to December 2012), at Benghazi Center of Infectious Diseases and Immunity (BCIDI) were collected from 750 HIV negative out-patients and 250 from HIV positive out-patients. These samples had been processed on cysteine lactose electrolytes deficient (CLED) agar (Oxoid, UK) with a standard loop and were incubated at 37°C overnight. The identification of all E. coli isolates were confirmed by API 20E (bioMerieux, France) and/or Phoenix™ Automated Microbiology System (Becton Dickinson, USA)(BD).

Antimicrobial susceptibility testing

E. coli isolates were tested for antimicrobial susceptibility by using the Kirby Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2008). The antibiotics were amoxycillin-clavulanic acid (30/10µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), imipenem (10 µg), aztreonam (30 µg), cefoxitin (30 µg), trimethoprim-sulfamethoxazole (25µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), ampicillin (10 µg), nitrofurantoin (300 µg) (Oxoid Ltd., Cambridge, UK). Antibiotic susceptibility testing was done.
on Mueller-Hinton agar (MHA) (BD). The results were recorded and interpreted as per CLSI (CLSI, 2008) recommendations. The E. coli ATCC 25922 strains was used as control.

Screening for ESBL producing isolates

All E. coli isolates were tested for ESBL production by their susceptibility to the third generation cephalosporins [ceftazidime (30 μg), cefotaxime (30 μg) and ceftriaxone (30 μg)] and aztreonam (30 μg) by using Kirby Bauer disk diffusion method (following the guidelines for CLSI, 2008). The isolates that showed inhibition zone ≤ 22 mm for Ceftazidime; ≤ 25 mm for ceftriaxone; and ≤27 mm for cefotaxime or aztreonam were considered to be probable producers of ESBL.

The isolates that showed resistance to at least one of the four antibiotics (ceftazidime, cefotaxime, ceftriaxone and aztreonam) were tested for ESBL production by the double-disk synergy test (DDST method) (Jarlier et al., 1988). E. coli ATCC 25922 was used as ESBL negative control and K. pneumonia ATCC 700603 was used as ESBL positive reference strain.

Screening for AmpC producing isolates: AmpC Disk Test

All isolates from E. coli (n=114) were screened by AmpC disk test. This test was performed as described by Singhal et al., (2005).

Modified three-dimensional test

AmpC enzyme production of all E. coli (n=114) isolates was detected by a modified three dimensional test (Manchanda & Singh, 2003). The growth was suspended in Buffered peptone water (Oxoid).

Statistical analysis

Statistical analysis was performed using SPSS software, version 8.0 (SPSS, Chicago, IL). The significance level was tested using t-test. A p-value < 0.05 was considered statistically significant.

Results and Discussion

A total of 1000 clean-catch midstream urine samples were collected from 750 HIV out-negative and 250 HIV positive out patients. 193(19.3%) yielded significant growth of a single organism. E. coli was the most common uropathogen, accounting for infections in 114 (59.06%) of the 193 patients. Of these 114 out patients, 27(23.68%) were HIV positive out-patients and 87(76.3%) were HIV-negative out-patients.

The antimicrobial susceptibility results of E. coli are summarized in Table 1. Imipenem was the only antibiotic effective against all these isolates (100%). Twenty seven of E. coli isolates from HIV positive patients showed susceptibility to cefoxitin, nitrofurantoin and ceftazidime (100%, 96.3% and 70.4%), respectively. These three antibiotics were the most effective against E.coli. Conversely, high resistance rates were detected for ampicillin (77.8%), nalidixic acid (59.3%) and amoxicillin-clavulanic acid (48.15%).

E. coli isolates from 87 HIV negative out-patients were highly susceptible to nitrofurantoin, ceftazidime, cefoxitin, ceftriaxone, aztreonam, cefotaxime and ciprofloxacin, (95.4%, 94.3%, 93.1%, 91.95%, 90.8 ,89.6% and 72.4), which
were the most effective drugs. Conversely, high resistance rates were detected for ampicillin (55.17%) and nalidixic acid (39.08%). There was no significant difference (p-value 0.071) between E. coli in HIV positive and negative patients in their resistance to different agents used in this study.

Overall 21.05% (24/114) of E. coli isolates were found to be ESBL producers whereas 78.9% (90/114) were ESBL non producers. 48.14 % (13/27) of HIV positive out-patients and 12.64% (11/87) of HIV negative out- patients were found to be ESBL producers (Table 2).

Table 3 shows antibiotics resistance of ESBL and non ESBL producing E. coli. ESBL producing E. coli in HIV positive out-patients showed maximum resistance to ampicillin (100%) and amoxicillin-clavulanic acid (84.6%); while no resistance was seen to nitrofurantoin (0%) and cefoxitin (0%). The non ESBL producing E. coli showed maximum resistance to ampicillin (57.1%) and nalidixic acid (50%); while the minimum resistance was seen with amoxicillin-clavulanic acid (14.4%); and non-resistance was seen with nitrofurantoin (0%) and cefoxitin (0%).

ESBL producing E. coli in HIV negative out- patients showed maximum resistance to ampicillin (100%),nalidixic acid (90.91%), ciprofloxacin (81.82%), trimethoprim-sulfamethoxazole (72.7%); and amoxicillin-clavulanic acid (72.7%); while the minimum resistance was seen with cefoxitin (18.18%) and non-resistance was seen with nitrofurantoin (0%). The non –ESBL producing E.coli showed maximum resistance to ampicillin (48.68%); while the minimum resistance was seen with cefoxitin (1.3%) and nitrofurantoin (3.94%). There was no significant difference (p-value 0.768) between ESBL producing E. coli in HIV positive and negative patients in their resistance to different agents used in this study.

Knowledge on local antimicrobial resistance trends among urinary isolates will help the clinicians to choose the appropriate empirical treatment of UTI (Randrianirina et al., 2007). This is the first study to detect AmpC production of E. coli causing UTI in BCIDI.

In this study the antibiotics resistance in HIV positive out-patients was more than...
in HIV negative out-patients except in cefoxitine, in which it was 0% in HIV-positive out-patients and 3.45% in HIV-negative out-patients. The Resistance to imipenem in both groups of out-patients was 0%. The high susceptibility to imipenem observed in our study is a clear indication that carbapenem resistance is still almost absent in E. coli isolated from UTI in the region. This can be explained by the infrequent use of imipenem in Benghazi. We found antibiotics resistance in general in HIV-positive out-patients and HIV negative out-patient increased from the results of our study in 2010 (Buzayan et al., 2010). In the present study, the highest antibiotics resistance in the E.coli strains from HIV positive and HIV negative out-patients was to ampicillin, nalidixic acid and trimethoprim-sulfamethoxazole.(77.8% vs.55.17%), (59.3% vs.39.08%) and (55.6% vs.36.8%).

Resistance of E.coli isolates from HIV positive patients to ampicillin was 77.8%. This result was similar to the results of other studies (Eryilmaz et al., 2010; Byam et al., 2010). Byam et al.(2010) found resistance to nalidixic acid was 20% which is less than 59.3% obtained in our present work; but the resistance rate to amoxicillin-clavulanic acid was 40% which similar to 48.15% found in our study. Also Byam et al.(2010) reported resistance rates 100% to trimethoprim-sulfamethoxazole and 10.0% to nitrofurantoin, which were higher than those we obtained of 55.6% and 0%, respectively.

Dash et al. (2013) study determined the susceptibility to antibiotics commonly used for the treatment of UTIs of E. coli urinary isolates obtained from outpatients in Odisha, India. Overall, resistance to ampicillin was 94.7%, followed by trimethoprim/sulfamethoxazole 51.9%, and amoxicillin-clavulanic acid 63.7%. The resistance rates found in our study were less than those in the Odisha, India study. In a study by Smet et al. (2010), the resistance rate reported was nalidixic acid 93.3%. This resistance rate was higher than that obtained in our work. Smets result for the resistance rate for amoxicillin-clavulanic acid was 26.7% similar to ours of 24.14%.

Even though nitrofurantoin is one of the oldest urinary anti-infective drugs in use, resistance to this drug remains minimal (Pourakbari et al., 2012). As seen in (Table 1), the overall resistance was 0% in HIV positive out-patients and 4.6% in HIV negative out-patients. Other studies of UTI from outpatients found resistance to nitrofurantoin at rates of 0% to 9.8% (Eryilmaz et al., 2010; Bosch et al., 2011; Dash et al., 2013). Muvunyi et al. (2011) reported a higher resistance rate 26.4% to nitrofurantoin.

Relative to our study of 2010, alarmingly higher rates of resistance to cephalosporins and aztreonam were found in our current study(22.2% to ceftazidime, 44.4% to cefotaxime and 40.7% to ceftriaxone and 33.3% to aztreonam) for HIV positive out-patients isolates. But in the HIV negative patients the resistance rates were low (5.7% to ceftazidime, 9.2% to cefotaxime, 8.05% to ceftriaxone and 9.2% to aztreonam). We observed increase in resistance rates to ciprofloxacin (27.6% in HIV negative and 44.4% HIV positive out-patients), because in the past few years ciprofloxacin have been prescribed more
Table 1. Antibiotics susceptibility pattern of *E. coli* in HIV positive and HIV negative out-patients

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>HIV positive out-patients (n=27)</th>
<th>HIV negative out-patients (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>NA</td>
<td>11(40.7%)</td>
<td>0</td>
</tr>
<tr>
<td>CIP</td>
<td>15(55.6%)</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>26(96.3%)</td>
<td>1(3.7%)</td>
</tr>
<tr>
<td>SXT</td>
<td>12(44.4%)</td>
<td>0</td>
</tr>
<tr>
<td>AMC</td>
<td>8(29.63%)</td>
<td>6(22.22%)</td>
</tr>
<tr>
<td>AMP</td>
<td>6(22.2%)</td>
<td>0</td>
</tr>
<tr>
<td>FOX</td>
<td>27(100%)</td>
<td>0</td>
</tr>
<tr>
<td>CAZ</td>
<td>19(70.4%)</td>
<td>2(7.4%)</td>
</tr>
<tr>
<td>CTX</td>
<td>15(55.6%)</td>
<td>0</td>
</tr>
<tr>
<td>CRO</td>
<td>15(55.6%)</td>
<td>1(3.7%)</td>
</tr>
<tr>
<td>ATM</td>
<td>16(59.3%)</td>
<td>2(7.4%)</td>
</tr>
<tr>
<td>IPM</td>
<td>27(100%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Sensitive. S; I, Intermediate; Resistant. R; Nalidixic acid, NA; Ciprofloxacin, CIP; Nitrofurantoin, F; Trimethoprim-sulfamethoxazole, SXT; Amoxicillin-clavulanic acid, AMC; Ampicillin, AMP; Cefoxitin, FOX; Ceftazidime, CAZ; Cefotaxime, CTX; Ceftriaxone, CRO; Aztreonam, ATM; Imipenem, IPM.

Table 2. Prevalence of ESBL, AmpC β-lactamase and Co-existence of resistance (ESBL + AmpC β-Lactamase) among *E. coli* in urine isolates.

<table>
<thead>
<tr>
<th>Resistance</th>
<th>HIV positive patients (n=27)</th>
<th>HIV negative out-patients (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL (%)</td>
<td>13(48.14%)</td>
<td>11(12.64%)</td>
</tr>
<tr>
<td>AmpC (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ESBL + AmpC</td>
<td>1(3.7%)</td>
<td>1(1.15%)</td>
</tr>
</tbody>
</table>

Table 3. Comparison of antibiotics resistance pattern in both ESBL producers and non ESBL producers of *E. coli*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>HIV positive out-patients (n=27)</th>
<th>HIV negative out-patients (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESBL producers (n=13)</td>
<td>Non ESBL producers (n=14)</td>
</tr>
<tr>
<td>NA</td>
<td>9(69.23%)</td>
<td>7(50%)</td>
</tr>
<tr>
<td>CIP</td>
<td>9(69.23%)</td>
<td>3(21.4%)</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SXT</td>
<td>9(69.23%)</td>
<td>6(42.9%)</td>
</tr>
<tr>
<td>AMC</td>
<td>11(84.6%)</td>
<td>2(14.3%)</td>
</tr>
<tr>
<td>AMP</td>
<td>13(100%)</td>
<td>8(57.1%)</td>
</tr>
<tr>
<td>FOX</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Nalidixic acid, NA; Ciprofloxacin, CIP; Nitrofurantoin, F; Trimethoprim-sulfamethoxazole, SXT; Amoxicillin-clavulanic acid, AMC; Ampicillin, AMP; Cefoxitin, FOX.
frequently for the empirical treatment of UTI in the region. Resistance rates for ciprofloxacin against UTI strains were reported as 5%, 15% and 31.9%, by Bosch et al. (2011), Eryilmaz et al. (2010) and Muvunyi et al. (2011), respectively. Recently, Dash et al. (2013) reported 53.4% resistance rates for *E. coli*.

On the basis of our results, antimicrobials such as ampicillin, ciprofloxacin, nalidixic acid, Amoxicillin-clavulanic and trimethoprim/sulfamethoxazole should no longer be recommended for initial empirical therapies for UTIs in Benghazi because of high resistance in *E. coli* that have ESBL producing in both HIV and non HIV out-patients.

The prevalence of the ESBL producers as in previous our study from BCIDI was reported to be 14.3% in HIV positive patients to 1.9 % in HIV negative out-patients. The occurrence of ESBL producers among *E. coli* in HIV positive patients in the current study was 13/27(48.14%), while 11/87(12.64%) of ESBL *E. coli* were found in HIV negative patients. The ESBL production which was reported among *E. coli* by Dalela et al. (2012) and Sasirekha (2013) were high with that which was found in our study 73.5% and 52.8%, respectively. Whereas Muvunyi et al. (2011) found ESBL of *E. coli* were 1.9 % in HIV negative out-patients. The ESBL production was more common in the *E. coli* isolates from HIV positive patient as compared to that in the *E. coli* isolates from HIV negative out-patient in this study.

In the present study, ESBL producing *E. coli* was found be multidrug resistant. In ESBL producing bacteria in HIV positive and HIV negative out patients have high percentage of resistant to ampicillin 100%. ESBL producing *E. coli* in both two groups of HIV positive and HIV negative out patients shows no resistance to nitrofurantoin, and minimum resistance to cefoxitin 0% and 18.18%, respectively. Resistance rate to Amoxicillin-clavulanic acid in HIV positive patients was higher than HIV negative patients 84.4% and 72.7%, while Resistance rates to nalidixic acid, ciprofloxacin and trimethoprim/sulfamethoxazole in HIV negative out-patients were higher than HIV positive out-patients.

Prevalence of AmpC β-lactamases among *E. coli* in the present study was found to be 0%, while Sasirekha (2013) and Rajni et al. (2008) found AmpC β-lactamases were 19.8% and 23% respectively. Cefoxitin resistance can be used to screen the isolates for detecting any possible AmpC β-lactamase production but can also indicate reduced outer membrane permeability (Thomson, 2001). Only one cefoxitin sensitive isolate of *E. coli* showed the production of AmpC β-lactamase along with the production of ESBL while 3 cefoxitin resistant isolates did not produce AmpC.

In this study, the co-existence of both AmpC β-lactamase and ESBL in *E. coli* has also been reported 3.7% (1/27) of the isolates in HIV positive out-patient and 1.15% (1/87) in HIV negative out-patients were found to have the co-existence. Dalela et al., (2012) and Sasirekha (2013) found 4/98(4.1%) and 8/91 (8.8%) of the co-existence among the *E. coli* isolates.

In conclusion, The ESBL producers are found in HIV positive out-patients more than in HIV negative out-patients. Nitrofurantoin was found to be the most effective antimicrobials. Our data analysis recommends nitrofurantoin as empirical
therapy against *E. coli* causing UTI. Antibiotics not tested in this study, such as fosfomycin, should be investigated for their potential in the future. Urine culture and antimicrobial susceptibility testing are recommended in BCIDI for treatment UTI patients.

**References**


Occurrence and detection of AmpC β-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at GurnTeghBahadurHospital, Delhi, India. J. Antimicrob. Chemother. 51: 415-418.


