Molecular Efflux pumps profiling of extended spectrum cephalosporin resistant Uropathogenic E. coli (ESC-UPEC)

Sahar Amer Ali¹, Hussein O.M. Al-Dahmoshi²*
¹Biology Department, college of science, University of Babylon, Iraq
   Email: amersahar575@gmail.com
   Mobile No.: +9647732951215
²Biology Department, college of science, University of Babylon, Iraq
   *Corresponding author Email: dr.dahmoshi83@gmail.com
   Mobile No.: +9647807771411

Abstract:

Resistance to beta-lactam antibiotics were common among enterobacteriaceae. Recently, *Escherichia coli* was one of the common uropathogen with incurable resistance to multiple cephalosporins. Resistance to extended spectrum cephalosporins like cefotaxime and ceftazidime can implicate chromosomal genetic treats like efflux pumps in such phenomenon. The current study aims to investigate efflux pumps gene profile among cefotaxime/ceftazidime resistant uropathogenic *E. coli*. Identification of *E. coli* was achieved by green metallic sheen on eosin methylene blue agar and confirmed by presence of *uidA* gene. Antibiotic resistance was studied using disc diffusion and biofilm formation by tissue culture plate method. Efflux pumps gene profiling include investigation of AcrAB-TolC, EmrAB-TolC, MacAB-TolC, EmrE and MdtK genes by polymerase chain reaction. The results revealed that 100%, 100%, 58%, 54%, 26%, 12% and 6% were resistant to cefotaxime, ceftazidime, cefepime, ceftriaxone, ciprofloxacin, levofloxacin and meropenem while all isolates were sensitive to imipenem. All isolates were biofilm former and possess all efflux pump genes except *acrB*. The current study conclude strong relationship between resistance to extend spectrum cephalosporin and efflux pump genes along with biofilm formation.

Keywords: extended spectrum cephalosporin, UPEC, efflux pump gene
Introduction:

Extended spectrum cephalosporin (ESC) specially from third generation like cefotaxime and ceftazidime were suitable and powerful drug of choice for different infections caused by Enterobacteriaceae [1-3]. Resistance to those 2 antibiotics may be not surprise especially in developing countries, especially middle east, due to established programs like stewardship of antibiotic resistance in governmental healthcare unites nor in private [4-6]. Additionally all antibiotics, without any exception, were over-the-counter (OTC). The overuse of cephalosporin in UTI treatment may be the main causes for emergence of extended spectrum cephalosporin resistance[7,8]. The extended spectrum cephalosporin resistance were common in Escherichia coli and Klebsiella pneumoniae[9,10]. The common resistance mechanisms for this phenomenon was producing of enzymes that disrupt the cephalosporin, especially extend spectrum beta-lactamases (ESBL). Many of the bacteria resistant to ESC are ESBL-producing organisms that are challenging to treat, with ESBL-producing Enterobacteriaceae being listed among serious threats related to antimicrobial resistance by the US CDC [11-13]. High level of resistance to cefotaxime and ceftazidime were shown in E. coli harboring blaTEM, blaSHV and blaCTX-M genes [14,15]. The aim of the current study little different seeking abut efflux pumps genes among Extended spectrum cephalosporin resistant UPEC.

Materials and Methods:

Bacterial isolates:

Fifty UPEC isolates were recovered from patients with cystitis whose visit urology consultant at governmental and private clinics at Hilla city, Babylon province, Iraq during a period of 6 months. All of isolates give pink colonies on MacConkey agar and green metallic sheen on eosin methylene blue agar plates.

Antibiotic susceptibility test

Antibiotic susceptibility test was performed according to CLSI-2021 using disc diffusion method on Muller-Hinton agar plates after standardization of inoculum for 0.5 McFarland [16].

Biofilm formation
The biofilm formation investigated according to tissue culture plate method (TCP described by Christensen et al., (1985)[17]. Also, the results interpreted according to Stepanovic et al., (2007)[18].

**Molecular study**

Bacterial DNA extraction were achieved using FavorPrep™ Blood / Cultured Cell Genomic DNA Extraction (Favorgen/Tiwan). Agarose gel electrophoresis were used to detect the DNA bands of extracted DNA using Redsafe DNA stain (Intronbio/Korea). All UPEC identification were confirmed using primer pairs for uidA gene [19]. Primer pairs were designed in this study to detect genes of efflux pumps for 5 classes, 3 tripartite pumps: AcrB-TolC (RND), EmrAB-TolC (MFS), MacAB-TolC (ABC) and 2 unipartite transporter: MdtK (MATE) and EmrE (SMR) table (1)

Table 1. Primer pairs sequence, annealing temperature and amplicon size

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence 5 to 3</th>
<th>Product bp</th>
<th>Annealing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>uidA</strong></td>
<td>F TGGTATTACCAGACGAAAACGGC</td>
<td>162</td>
<td>62°C, 30 sec.</td>
</tr>
<tr>
<td></td>
<td>R ACGCGTGTTACAGTCTTGGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AcrA</strong></td>
<td>F ATCACCTTTCGACTGTGTTG</td>
<td>256</td>
<td>58.3°C, 30 sec.</td>
</tr>
<tr>
<td></td>
<td>R CGACAAACAGGCCCACAAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AcrB</strong></td>
<td>F CATAAAACACGCCCCTGGTCCT</td>
<td>432</td>
<td>60.3°C, 30 sec.</td>
</tr>
<tr>
<td></td>
<td>R GCTACCCGTAAGTCGATGGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TolC</strong></td>
<td>F CGATCGTGATGCTGCTTBTG</td>
<td>432</td>
<td>58.3°C, 30 sec.</td>
</tr>
<tr>
<td></td>
<td>R GCTACCCGTAAGTCGATGGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>emrA</strong></td>
<td>F CGCTGAGGCTGACTCTGTATG</td>
<td>287</td>
<td>58.3°C, 30 sec.</td>
</tr>
<tr>
<td></td>
<td>R ATTTTGCCTGGAAGCAGTAGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>emrB</strong></td>
<td>F CTGCGCCGCTAGGATTTATT</td>
<td>59.3°C, 30 sec.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R ATCCCAAGCCCTTCCAGTTG</td>
<td>436</td>
<td></td>
</tr>
<tr>
<td><strong>EmrE</strong></td>
<td>F ACACGGTTATGGCCATCTGT</td>
<td>57.2°C, 30 sec.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R ATGTGGTGGTTGCTCCTGACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MacA</strong></td>
<td>F GCACAACAAGGACCCGACCAT</td>
<td>225</td>
<td>58.3°C, 30 sec.</td>
</tr>
<tr>
<td></td>
<td>R CATATCCAGGCCGACGCAAAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MacB</strong></td>
<td>F GCATGAGGGCGCATGATTATT</td>
<td>364</td>
<td>57.2°C, 30 sec.</td>
</tr>
<tr>
<td></td>
<td>R AAACGCGTGAGGTCCTGATTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MdtK</strong></td>
<td>F CTCTTGCTACGAGGACTGCT</td>
<td>350</td>
<td>59.3°C, 30 sec.</td>
</tr>
<tr>
<td></td>
<td>R TTCACCGGGATGTTCCACCAG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results:

The antibiotic susceptibility test for cefotaxime, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, levofloxacin, imipenem and meropenem were investigated. The results shown that, all isolates 50(100%) were resistant to cefotaxime and ceftazidime, 29(58%) and 27(54%) were resistant to cefepime and ceftriaxone respectively. Resistance to ciprofloxacin was 13(26%), levofloxacin was 6(12%), meropenem was 3(6%) while all isolates were sensitive to imipenem (figure 1). All 50 isolates of UPEC confirmed on EMB agar with green metallic sheen give positive results for uidA gene amplification (162 bp) band (Figure 2A).

Investigation of efflux pumps gene revealed that AcrAB-TolC genes present in acrA 50(100%) acrB 43(86%) and tolC 50(100%) (figure 2B-2D). EmrAB-TolC present in emrA 50(100%) and emrB 50(100%) (figure 2E,2F). EmrE found in emrE 48(96%) while MacAB-TolC present in macA 50(100%) and macB 49(98%) (Figure 2G-2I). MdtK were present in 50 (100%) (figure 2J).

Results of biofilm formation revealed that all 50(100%) of UPEC isolates were biofilm former. Relationship between antibiotic resistance and biofilm formation with existence of efflux pumps gene were shown in table (2). Approximately all isolates have all efflux pump genes under study except for acrB

![Figure 1. Antibiotic resistance of UPEC](image-url)
Figure 2. Agarose-Gel electrophoresis (1.5% in TBE) for amplicon of: A) 162bp for *uidA*, B) 256bp for *acrA*, C) 432bp for *acrB*, D) 596bp for *tolC*, E) 287bp for *emrA*, F) 436bp for *emrB*, G) 249bp for *emrE*, H) 225bp for *macA*, I) 364bp for *macB*, J) 350bp for *mdtK*.

Figure 3. Biofilm formation and efflux pump genes among cefotaxime/ceftazidime resistant UPEC

Discussion:
The results show presence of all efflux pumps classes in approximately all UPEC isolates. Schuster et al., (2017)[20] found that efficacy of AcrAB-TolC mediated broad-spectrum drug efflux, including agents primarily developed for Gram-positive pathogens, in a clinical isolate representative of a globally–emerging lineage. Additionally Eshaghi et al. (2021) found that presence of acrAB, emrAB, and mdtk efflux pumps genes is potentially a factor in resistance to fluoroquinolone antibiotics[21]. Significant correlation between the presence of the *acrAB-tolC* genes and the resistance to nalidixic acid, ofloxacin, and
piperacillin-tazobactam was observed[22]. AcrAB-TolC was the predominant efflux pump for most of antibiotic classes[23]. EmrAB-TolC was also stated as more prevalent efflux pump of UPEC[24]. EmrE effluxes a wide variety of aromatic cation antibiotics through the inner membrane of Escherichia coli using the proton motive force, conferring resistance to antibiotics with this chemical profile [25). In E. coli and other Gram-negative bacteria, including pathogenic organisms, the MacA-MacB-TolC assembly (hereafter, MacAB-TolC) leads to drug resistance and virulence phenotypes. These pumps are responsible for the transport of outer membrane glycolipids[26]. The mdtK of the MATE type also contributed to multidrug resistance[27] This group of bacteria's biofilm-forming capacity leads them to stick together and get embedded in a matrix of extracellular polymeric substance known as exopolysaccharide. This generates a protective environment that makes antibiotic penetration more difficult and protects against insults like dehydration and food scarcity[28]

**Conclusions:**

The current study conclude strong relationship between resistance to extend spectrum cephalosporin and efflux pump genes along with biofilm formation.

**References:**


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