Detection of blaCTX-M gene among Pseudomonas aeruginosa isolated from water samples in Baghdad

Saba R. Khdaier¹, Ali M. Hassan¹, H. M. Abdul Wahab², Nibras N. Mahmood¹
¹Department of Biology, College of Science, Mustansiriyah University, IRAQ.
²Institute of Technology, IRAQ.
*Correspondant email: Sabaalaltaii@yahoo.com

Abstract
A total of 50 environmental Pseudomonas aeruginosa isolates were collected from sewage and tap water in Baghdad, Iraq. The MICs of Cefotaxime and Ceftazidime were determined by using agar dilution method, The MIC ranged from 2 to 256 µg/ml. The results of antibiotic sensitivity test showed that among sewage P. aeruginosa isolates, resistance was observed most often to Ticarcillin (92%), Penicillin G (84%), Ceftazidime (12%), (8%) for each of Cefotaxime and Ticarcillin. On the other hand, all tap water isolates were sensitive to Ofloxacin and Levofloxacin, Except (5%) of isolates were resistant to Cefotaxime (25%) to Ceftazidime and (95%) to Ticarcillin. All isolates were tested for Extended-Spectrum β-Lactamase (ESBL) production. Ten isolates (20%) were found to be ESBL producers. All environmental P. aeruginosa isolates were screened for the presence of the blaCTX-M genes by application PCR, Only (30%) of them were positive for this test.

Keywords: Pseudomonas aeruginosa, Sewage, blaCTX-M gene.

Introduction
Pseudomonas aeruginosa is a pathogenic bacterium that has been thoroughly investigated since the 19th century and is generally regarded as a freshwater or terrestrial organism, this bacterium has a remarkable ability to adapt and thrive in a variety of environments: water [1]. Soil occupational places, such as M. et al. Working fluids, hospital and municipal wastewater, tap water and water distribution systems [2] [3]. Multidrug-resistant (MDR) strains of P. aeruginosa are isolated from patients suffering from nosocomial infections. Thus infections are particularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs [4]. Resistance to β-lactam antibiotics dates back to the first years of discovery of resistance to the first antibiotic, penicillin. The first β-lactamase was observed in Escherichia coli bacteria which hydrolyzed penicillin [5].

In recent years, a new family of plasmid-mediated ESBLs, called CTX-M (Cefotaxime-hydrolyzing β-lactamase), has been arisen that preferentially hydrolyzed Cefotaxime. CTX-M was reported in 1989 for the first time in Germany, and is often found in E. coli and
Klebsiella pneumoniae as well as in other Enterobacteriaceae [6]. In recent years; CTX-M-type β-lactamases have been recognized as a growing family possessing a high level of hydrolyzing activities, especially against Cefotaxime (CTX) and Ceftriaxone. Nearly 40 variants of the CTX-M-type enzymes have been identified and registered to date [7][8]. The aims of this work were to detection of bla CTX-M among Environmental P. aeruginosa isolates in Baghdad.

Materials and Methods

**Bacterial Isolates**

A total of 50 isolates of *P. aeruginosa* bacteria were collected from sewage (Al-Rustamiyah) and tap water (Palestine Street) in Baghdad. The isolates were identified by their colony characteristic, Gram-stain and confirmed by using Vitec 2 system [9].

**Antibiotic Susceptibility Testing**

The antibiotic susceptibility test was done by using Kirby-Bauer disc diffusion technique on Mueller Hinton agar (Oxoid, England) following Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. Isolates were tested against the following antimicrobial agents: Cefotaxime, Ticarcillin, Cefepime, Penicillin G, Aztreoname, Meropenem, Ceftazidime, Ofloxacin and Levofloxacin. The results compared with standard strain *P. aeruginosa* ATCC 154427 from biology laboratory, Mustansiryah University.

**Minimal Inhibitory Concentrations**

The MICs of Cefotaxime and Ceftazidime were determined by using Mueller-Hinton agar with antibiotic concentrations ranged (2-512) μg/ml according to the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI) document. ESBL production by Combined disk test CDT (Phenotypic confirmatory test): A disk of Ceftazidime (30μg) alone and a disk of Ceftazidime + Clavulanic acid (30μg/10μg) were placed independently, 30mm apart, on a lawn culture of 0.5 Mc-Farland opacity of the test isolate on Mueller Hinton Agar (MHA) plate and incubated for 18-24 hours at 35°C. An increase of ≥5mm zone of inhibition diameter around the Ceftazi-dime/Clavulanic acid in comparison to Ceftazidime confirmed ESBL production [11, 12].

**DNA Preparation and PCR**

A PCR reaction with specific primers was performed to identify bla CTX-M gene of each environmental isolate (Table 1) according to Shacheraghi *et al.* [13]. DNA template was prepared as described by Olsvik *et al.* [14]. (25μl) of PCR amplification mixture contained deionized sterile water (12.5μl) Green Go Taq Master Mix pH [8] (Promega, USA).

<table>
<thead>
<tr>
<th>Primer type</th>
<th>Primer sequence</th>
<th>Product size bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward primer bla CTX-M</td>
<td>CGCTTTTGCATGTGCAG</td>
<td>550</td>
</tr>
<tr>
<td>Reverse primer bla CTX-M</td>
<td>ACCCGCATATCGTTGGT</td>
<td></td>
</tr>
</tbody>
</table>

*The protocol for the PCR condition was: 94°C for 45 s, 53°C for 45 s, and 72°C for 60 s, with a cycle number of 32, Gradient PCR (TechNet – 500, USA).*

**Dendrogram Construction and Genetic Relatedness**

Dendrogram for cluster analysis of all the isolates were subjected to evaluation. The dendrogram was constructed on the basis of the banding pattern produced. A binary table or a haplotype matrix for each isolate was constructed by linearly composing presence (1) and absence (0), data derived from analysis of the gel/antibiogram was subjected for statistical analysis by squared. Euclidean distance is using the software study.

**Statistical Analysis**

This enabled the plotting of dendrogram showing the level of genetic similarity among the isolates.

**Results and Discussion**

A total of 50 environmental *P. aeruginosa* isolates were collected from sewage and tap water in Baghdad, Iraq. Table 2 shows the large scale distribution of resistant of bacteria to antibiotics in water is broad applications of antibiotics by humans. Persistence of antimicrobial resistant organisms is a growing public health problem in aquatic ecosystem [15]. The
The public health significance of a large number of Pseudomonas bacteria found in water is not very clear usually Pseudomonas spp. isolated from water are resistant to antimicrobials [16].

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sewage water</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Tab water</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

The MICs of Cefotaxime and Ceftazidime were determined using the agar dilution method. MICs of Cefotaxime and Ceftazidime were determined using agar dilution method, which ranged between 2-256µg/ml. Results of Antibiotic sensitivity showed that among sewage P. aeruginosa isolates, resistance was observed most often to Ticarcillin (92%), Penicillin G (84%), Ceftazidime (12%), (8%) for each of Cefotaxime and Ticarcillin, on the other hand all tap water isolates were sensitive to(Ofloxacin and Levofloxacin, 5%) of isolates were resistant to Cefotaxime and (25%) to Ceftazidime and (95%) of Ticarcillin (see Table 3). All isolates were tested for ESBL production. Just 10 isolates (20%) were found to be ESBL producers.

<table>
<thead>
<tr>
<th>Antimicrobial Agents</th>
<th>Resistance % of Sewage</th>
<th>Resistance % of Tap water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>Cefepime</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>84</td>
<td>90</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>Meropenem</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Bali et al. [17], mentioned that ESBLs isolates have able to hydrolyze 3rd and 4th generation cephalosporins and monobactam and the percentage of ESBLs-producing isolates of G-ve were 5.2 %. Bacterial species that produce ESBL is an important threat to clinical therapeutics. These organisms elaborate plasmid-encoded β-lactamases, a variety of which have been described among members of the family Enterobacteriaceae. First described in 1983, ESBL producers have contributed to the dramatic increase in recent years to resistance among gram-negative bacteria to β-lactam agents. Plasmid-borne genes code for enzymes that hydrolyze Penicillins, Cephalosporins, and Aztreonam are inhibited by Clavulanic acid [18]. All Environmental P. aeruginosa isolates were tested for the presence of the blaCTX-M genes by PCR, 30% of P. aeruginosa isolates were positive most of them were isolated from sewage (Fig 1).

CTX-M β-lactamases are a class of β-Lactamase which have been more recently recognized to preferentially hydrolyze Cefotaxime and were initially reported in the second half of the 1980s. Theses enzymes (Cefotaximases) are a relatively novel family of plasmid mediated extended spectrum Cephalosporins and have been classified under Ambler class [19]. Unlike that of TEM or SHV type ESBLs, the population stricter of CTX-M producing isolates is complex and associated with the spread of specific plasmids and/or epidemics. CTX-M type ESBLs have become widely dispersed in many parts of the world and these resistance to Cefotaxime than to Ceftazidime.

Study of Al-Kaabi [20] in Baghdad mentioned that the percentage of bla CTX-M gene was 83.3%, while blaCTX-M gene appeared in all isolates (100%) in P. aeruginosa isolates. In the same manner, Al-Margani et al. (2013) reported that all of ESBLs and MBLs producer P. aeruginosa isolates carried bla CTX-M gene [21]. Al-Margani (2014) showed that the presence of qnr gene in environmental P. aeruginosa isolates in Baghdad [22].

Dendrogram for cluster analysis of all the isolates were constructed using dice coefficient values there were two major clusters when dendrogram was generated on the basis of their bla CTX-M genes (A, B) among B group the isolates clusters in to two subgroups (B1 and B2) dendrogram shown 10 isolates unique pattern.
These results indicated that the appropriate of dendrogram as a tool for discrimination isolates and to give idea about different same species isolates from the same sources of isolation. This results agreed with Jacome et al. (2011) showed molecular typing for the identification of clonal relationship between isolates of P. aeruginosa. The latter has been quite frequented in the recent years for simplicity and efficiency [23]. A close genetic relationship among isolates showed the distribution of organisms in the environment studied. Although some isolates belonged to different clustering groups, they showed more than 80% similarity indicating these isolates originated from a limited number of primary clones. These isolated might tolerate genetic divergence arising from point mutation, insertion or deletion of chromosomal DNA.

References


