Characterization of Ampicillin Resistant Gene (blaTEM-1) Isolated from E. coli in Northern Jordan

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Abstract

The aim of this project is to study the molecular characteristics of blaTEM-1 gene, and the associated ampicillin resistance mechanisms present in E. coli in north Jordan. In this study, 150 unrelated Escherichia coli bacterial samples were isolated from different clinical sources (urine, blood, pus and abscess) and tested for their susceptibility to 17 antimicrobial agents, including ampicillin. The isolates were typed by plasmid profiling, and were investigated by PCR for the presence of various resistance genes. Out of the 150 isolates, 14 strains were multi-resistant; they showed resistance to Cotrimoxazole (70%), Ampicillin (67%), nalidixic acid (51%), Cephalothin (27%), Augmentin and Nitrofurantoin (19%), Tetracycline and Ciprofloxacin (15%) and Gentamycin (12%). Plasmid analysis of clinical isolates showed the presence of 1 to 7 plasmids with size ranging from 1.9 to 21.1 Kb compared with the control E.coli ATCC 25922 (size range from 2 to 19.5 Kb). PCR results showed the presence of blaTEM-1 gene which was responsible for Ampicillin resistance in 5 of the 14 isolated E. coli strains; the gene was located on a plasmid having a size of 1190 bp. This is the first study describing the presence of the blaTEM-1 gene in bacterial isolates in Northern Jordan. The blaTEM-1 gene found in this plasmid showed strong correlation between genotype conferred resistance determined by PCR and antibiotic susceptibility patterns.

Keywords: blaTEM-1 gene, antimicrobial resistance, PCR, resistant plasmid Bulinus species.

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Introduction

Escherichia coli (E. coli) are considered one of the main causes of nosocomial infections in humans. It’s also a common inhabitant of human and animal guts [1], and it’s considered an indicator of fecal contamination in water and food. Pathogenic variants cause intestinal and extra intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicemia [2,3]. The widespread use of antibiotics could be associated with the selection of antibiotic resistance mechanisms in pathogenic and non-pathogenic isolates of E.coli [4]. Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries [5-7]. The spread of resistance genes through plasmid transfer plays an important role in the dissemination of resistance genes in Gram-negative enteric pathogens [8]. Routine monitoring of antibiotic resistance tends to help in providing information on antibiotic therapy and resistance control [9]. Normal intestinal flora is a reservoir for resistance genes, the prevalence of resistance in commensal E. coli is reported to be a useful indicator of antibiotic resistance in bacteria in the community [10]. The emergence of multi-resistant Escherichia coli has been previously reported in humans and in different animal species increasing the public health concern [11]. On the other hand, the production of extended spectrum beta-lactamases (ESBLs) by Enterobacteriaceae, specifically by E. coli, has caused a major concern in several countries, being frequently implicated in human infections. Previous reports have described ESBL-containing E. coli strains in healthy animals [12-14]. Studies involving E. coli are of particular relevance because these species can occupy multiple niches, including human and animal hosts [15]. In addition, E. coli strains can exchange efficiently genetic material with pathogens such as Salmonella, Shigella, Yersinia, and Vibrio species, as well as with pathogenic E. coli [16,17].

Resistance to β-lactam antimicrobial agents in E. coli is primarily mediated by β-lactamases, which hydrolyze the β-lactam ring and thus inactivate the antibiotics [18]. Several
different β-lactamases have been described; over 200 β-lactamases have been classified into four main groups and eight subgroups according to their functional and structural characteristics [19-21]. It has been reported that TEM-, SHV- and OXA-type β-lactamases are the most predominant ones [22]. In a study done on Portuguese Cheese, 85% of the isolates identified belonged to the Enterobacteriaceae family. The presence of the blaTEM gene was detected in 80.9% of the tested isolates [23].

The present study aims to study the molecular characteristics of blaTEM-1 gene, and the associated ampicillin resistance mechanisms present in E.coli in northern Jordan.

Methods

Bacterial isolates

One hundred and fifty unrelated E. coli bacterial samples were isolated from different clinical sources (urine, blood, pus and abscess) from Princess Rahma Hospital, Princess Basma Hospital, Yarmouk Clinic and King Abdullah Hospital in Irbid area in North Jordan. The isolated bacteria were confirmed by direct microscopic examination based on Gram stain, and standard biochemical tests [24].

Antimicrobial susceptibility test

The antibiotic sensitivity testing of bacterial isolates was analysed according to Kirby-Bauer standard single disc diffusion method [25] and the clinical laboratory standard institute [26] on Mueller Hinton Agar plates. Seventeen antimicrobial drugs, including ampicillin, were tested (Table 1). The size of the area of suppressed growth (zone of inhibition) was determined by the concentration of the antibiotics present in the area, and therefore, the diameter of the inhibition zone denotes the relative susceptibility to a particular antibiotic. The interpretation of the results as sensitive or resistant was determined according to standard charts provided by the manufacturers. The bacterial strain E.coli ATCC 25922 was used as control.

Table 1: The antibiotics and the concentrations used for Sensitivity testing of E. coli samples, following the disc method.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Symbol</th>
<th>Concentration (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>AMK</td>
<td>30</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>AMP</td>
<td>10</td>
</tr>
<tr>
<td>Augmentin</td>
<td>AUG</td>
<td>30</td>
</tr>
<tr>
<td>Azteronam</td>
<td>AZT</td>
<td>30</td>
</tr>
<tr>
<td>Cefamandole</td>
<td>CMD</td>
<td>30</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>CTX</td>
<td>30</td>
</tr>
<tr>
<td>Cefprozil</td>
<td>CPR</td>
<td>30</td>
</tr>
<tr>
<td>Ceftriaxime</td>
<td>ZOX</td>
<td>30</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>KF</td>
<td>30</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>SXT</td>
<td>25</td>
</tr>
<tr>
<td>Imipenem</td>
<td>IMP</td>
<td>10</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>GN</td>
<td>30</td>
</tr>
<tr>
<td>Nalidexic acid</td>
<td>NA</td>
<td>30</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>NIT</td>
<td>300</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>NOR</td>
<td>10</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>PIP</td>
<td>100</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>RIF</td>
<td>5</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>TCP</td>
<td>30</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TET</td>
<td>30</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>TOB</td>
<td>10</td>
</tr>
</tbody>
</table>

Plasmid isolation

The selected bacterial strain (single colony) was grown overnight in Luria-Bertani (LB) broth at 37°C with aeration using an orbital shaker. The plasmid DNA was then extracted from lysed E.coli cells using a Plasmid Miniprep kit from Promega Corporation (USA).

Detection for the presence of blaTEM-1 β-lactamases causing resistance to Ampicillin by PCR

PCR analysis was used to detect the presence of blaTEM-1 gene (Promega-USA), with the primers: 5’ – TTC TTG AAG ACG AAA GGG C- 3’ (size 19 b) and 5’ - ATG GTG AGT GAT GGA ACG AAA AC- 3’ (size 20 b). Primers and amplification conditions for blaTEM were described previously [27]; the size of the amplified product was 1190bp. Amplified DNA products were resolved by conventional electrophoresis through horizontal 0.8% agarose gel (Scie-Plas limited, Southam, Warwickshire, United Kingdom) containing ethidium bromide with 0.5X Tris-Borate-EDTA buffer at 150v; the results were visualized and photographed under a UV light. The approximate size of plasmids (in bases) was determined by comparing them to a standard marker Lambda DNA Hind III digest (Promega-USA). 16S rRNA primers were used as internal control for all PCRs [28]. The following primers were used 5’- GGA GGA AGG TGG GGA TGA CG- 3’ (size 20 b) and 5’- ATG GTG TGA CGG GCG GTG TG – 3’ (size 20 b); the size of the amplified product was 241 bp. PCR was performed on a Gene Amp PCR system 9700 (Perkin-Elmer Crop/ Applied Biosystems Division). Amplification conditions were 30 cycles of 94°C for 60 seconds, 60°C for 60 seconds, and 72°C for 60 seconds, with a final extension of 72°C for 5 minutes.
Results

Antimicrobial susceptibility test

All isolates were from human origin (urine, blood, pus and abscesses). The E. coli strains isolated from the clinical samples showed resistance mostly to Cotrimoxazole (70%), Ampicillin (67%), Nalidixic acid (51%), Cephalothin (27%), Augmentin and Nitrofurantoin (19%), Tetracycline and Ciprofloxacin (15%), and Gentamycin (12%) (Figure 1).

Plasmid profile

Plasmid analysis of the clinical isolates showed the presence of 1 to 7 plasmids per cell with size range from 1.9 to 21.1 Kb as compared with the control E. coli strain ATCC 25922 which was found to contain 6 plasmids with sizes ranging from 2.0 to 19.5 bp (Table 2 and Figures 2 and 3).

Table 2: Plasmid characterization. The isolated plasmids from E. coli strains, showing numbers, sizes and resistance to antibiotics.

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Clinical Source</th>
<th>Number of Plasmids Isolated</th>
<th>Size of Plasmid (Kb)</th>
<th>Resistant Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urine</td>
<td>5</td>
<td>21.0, 15.0, 5.0, 4.2</td>
<td>AMP, NA, SX, KF</td>
</tr>
<tr>
<td>2</td>
<td>Swab</td>
<td>5</td>
<td>21.0, 14.5, 5.0, 3.5</td>
<td>TCP, TET, IMP</td>
</tr>
<tr>
<td>3</td>
<td>Pus</td>
<td>2</td>
<td>21.0, 3.5</td>
<td>TCP, TET, IMP</td>
</tr>
<tr>
<td>4</td>
<td>Abscess</td>
<td>4</td>
<td>21.0, 17.0, 9.0, 4.3</td>
<td>AMP, CIF, NA, KF</td>
</tr>
<tr>
<td>5</td>
<td>Urine</td>
<td>4</td>
<td>21.5, 19.0, 4.3, 2.0</td>
<td>AMP, CIP, NA, NOR, CXM</td>
</tr>
<tr>
<td>6</td>
<td>Swab</td>
<td>7</td>
<td>21.0, 7.5, 4.0, 4.2,</td>
<td>AMP, SX, NA, CXM</td>
</tr>
<tr>
<td>7</td>
<td>Urine</td>
<td>4</td>
<td>21.0, 15.0, 5.0, 1.9</td>
<td>AUG, CAZ, AMP, NOR, SX, NIT</td>
</tr>
</tbody>
</table>

Figure 1: Percentage resistance to the antibiotics used against E. coli bacteria in this study following the susceptibility test.

Figure 2: Agarose gel electrophoresis showing the plasmids isolated from E. coli in all the samples. M: Lambda DNA EcoR1 + HindIII Markers. (A); Lane 1: E. coli ATCC 25922, Lane 2: E. coli isolated from Abscess (princess Rahma Hospital), Lane 3: E. coli isolated from Urine (princess Rahma Hospital), Lane 4: E. coli isolated from Swab (Yarmouk clinic), Lane 5: E. coli isolated from Urine (King Abdullah Hospital), Lane 6: E. coli isolated from Urine (Princess Basma Hospital), Lane 7: E. coli isolated from Blood (princess Rahma Hospital), Lane 8: E. coli isolated from Swab (Princess Basma Hospital), Lane 9: E. coli isolated from Abscess (princess Basma Hospital), Lane 10: E. coli isolated from Urine (Yarmouk clinic), Lane 11: E. coli isolated from Pus (Princess Basma Hospital), Lane 12: E. coli isolated from Urine (Yarmouk clinic), Lane 13: E. coli isolated from Pus (Princess Basma Hospital), Lane 14: E. coli isolated from Swab (King Abdullah Hospital), Lane 15: E. coli isolated from Swab (King Abdullah Hospital), Lane 16: E. coli isolated from Pus (King Abdullah Hospital).
Clinical isolates of E. coli from different sources of infection were investigated for the presence of β-lactamase-encoding genes. PCR and DNA plasmid profiling showed that the ampicillin resistance was due to the presence of a TEM-1 β-lactamase gene. TEM-1 is the most commonly encountered β-lactamase in Gram-negative bacteria, and up to 90% of ampicillin resistance in E. coli is due to the production of TEM-18. This is the first study to be conducted in northern Jordan that discusses the association between the presence of TEM β-lactamase gene and ampicillin resistance by E.coli.

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**References**

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