MOLECULAR DETECTION OF ANTIBIOTIC RESISTANCE GENES (TEM, GES AND OXA) IN UROPATHOGENS

Fatima Hamza Alzubaidy Taif Al-saadi and Najlak Taklef

ABSTRACT:

Antibiotic resistance is one of the most severe global public health threats; it can kill 700,000 people and could rise to ten million by 2050 so the study includes detection the antibiotic resistant, the infection of the urinary tract is one of the predominant infections among people that result from pathogenic bacteria. More than 80% of UTI instances are brought on by a bacterium, and the most common culprit is E. coli, which is a typical component of the intestinal flora. The most common gram-negative bacterium in the study is E. coli about 25%, K. pneumoniae about 12%, Enterobacter aerogenes about 10% and others while the most common gram-positive bacteria that cause UTI is S. aureus 12%, S. epidermidis about 8% and Enterococcus faecalis about 4%. The bacterial isolates were discovered by morphological, biochemical and VITEK-2 compact system. The study includes detection the antibiotic-resistant by VITEK-2 compact system and the antibiotic-resistant gene for detect bacteria that contain the most antibiotic-resistant gene and eventually lead to modification in the future. The gene used in the study is (TEM found at 41%, GES at 41% , and OXA at 50%) the three resistant genes found in K. pneumoniae, E. coli and P. aeruginosa but the other bacteria also contain one of the tested genes such as TEM found in K. oxytoca and P. mirabilis, GES in Enterobacter aerogenes and Citrobacter freundii, while the OXA gene in A. baumanii, C. freundii and K. oxytoca.

Keywords: Carbapenemase gene, B-lactamase gene, UTI. Citrobacter freundii, Enterobacter aerogenes.

INTRODUCTION:

Urinary tract infection (UTI) refers to broad array of clinical problems, from kidney infection and sepsis to bacteriuria without symptoms, and it is a primary reason of human disease in the United States, costing an estimated $1.6 billion each year(1).

UTIs are brought on by bacteria. in more than 80% of cases; the most common organism responsible for this bacterium is E. coli, which is found in the natural flora of the intestine(2). E. coli, Pseudomonas species, Klebsiella species, Enterobacter species, Acinetobacter species, and Citrobacter species are the Gram-negative bacteria that are most common. Gram-positive bacteria were the most common type, with Enterococcus species being the most common and having a high prevalence of vancomycin-resistant enterococci; Staphylococcus species and Streptococcus species were the next most common (3).

Antibiotic resistance is one of the world's most serious public health issues, with the potential to kill 700,000 people by 2050 (4). When a microbrial strain becomes resistant to medications, it is known as antimicrobial resistance (AMR), can prevent or halt their growth and make them resistant to medical interventions. In the past twenty years, a variety of AMR infections have emerged worldwide. Mycobacterium tuberculosis that
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is both extensively and multidrug resistant (MDR, XDR-MTB), as well as methicillin-resistant Mycobacterium tuberculosis (MDR, XDR-MTB), as all ESBL-producing bacteria (MTB), S. aureus is a bacterial species (MRSA)\(^5\).

One of the most popular routes by which resistance to B-lactam antibiotics is established is through extended-spectrum B-lactamases (ESBLs)\(^6\). These B-lactamases are plasmid-encoded and have been identified in clinical isolates of Enterobacteriaceae, including K. pneumoniae, Pseudomonas aeruginosa, and E. coli\(^7\). The most prevalent ESBL types among clinically significant Enterobacteriaceae species are undoubtedly the TEM (temoniera), SHV (sulphydryl-variable), and CTX-M (cefotaximase) proteins; nonetheless, the existence of less well-researched ESBL types, like OXA (oxacillinase) and GES (Guyana extended spectrum -lactamase), has been observed\(^8\).

Recent research suggests that the proliferation of genes encoding ESBLs may contribute to the development of resistance to antibiotics and complicate the treatment of P. aeruginosa infections due to limited therapeutic options\(^9\). Unlike TEM and SHV enzymes, OXA-type enzymes are part of a newly emerging family of ESBLs that are members of molecular class D and functional group 2d. Pseudomonas aeruginosa is the organism that produced the first B-lactamase enzymes\(^10\).

Carbapenems remain the last-line antimicrobials for treating multidrug-resistant microorganism infections. Thus, the emergence and spread of carbapenemase-producing organisms (CPOs) represent a global health threat because they are associated with limited treatment options and poor clinical outcomes. Clinically important carbapenemases belonging to different Ambler classes include class A carbapenemase Klebsiella pneumoniae carbapenemase (KPC) type, class B metallo-β-lactamases IMP-type metallo-β-lactamase (IMP) type, Verona integrin-encoded metallo-β-lactamase (VIM) type, and New Delhi metallo-β-lactamase (NDM) type, and class D carbapenemase OXA-48 like. Among the class A β-lactamases of the Guiana extended spectrum β-lactamase (GES) type, those with an amino acid substitution of Gly170Ser within the Ω-loop region, such as GES-24 and GES-5, exhibit carbapenem-hydrolyzing activities. The relatively low carbapenem minimum inhibitory concentrations (MICs) of some isolates of GES carbapenemase producers in combination with the lack of available selective inhibitors specific for GES carbapenemases may make it difficult to detect these enzymes, leading to an underestimation of the true prevalence of GES carbapenemase-producing isolates. These carbapenemase genes are often located in mobile genetic elements (MGEs) such as plasmids, transposons, and integrons, thereby facilitating their rapid spread among Enterobacterales and other Gram-negative bacteria\(^11\). The acquisition of MGEs by high-risk bacterial clones with adaptive traits in humans and environments with accumulating virulence and resistance genes plays critical roles in the successful dissemination of carbapenemase genes\(^12\). The geographic distribution of CPOs is variable. Generally, the high endemicity of certain carbapenemases is associated with specific regions or countries, such as the KPC type in the USA, Israel, Greece, and Italy; NDM type in the Indian subcontinent; OXA-48 like in Turkey, the Middle East, and North Africa; and IMP type in East Asian countries including Japan\(^13,14\). Moreover, several factors, such as international travel/migration, repatriation of patients, food import, and wildlife migration from areas of high endemicity, can accelerate the extensive spread of CPOs to neighboring regions, surrounding countries, and other continents\(^15–18\), leading to constant changes in local and global epidemiology.
MATERIAL AND METHODS:

Collection and identification of samples:

At Al-Sader medical city in Al-Najef, one hundred urine samples from patients with UTIs were gathered and placed in sterile tube containers with labels. After collecting the urine sample, it was cultured for 1 day at 37 °C on nutritional agar, MacConkey agar, and chromogenic agar \(^{19}\). The biochemical test was also utilized for identification, and the procedure was finished using the Vitek-2 compact system \(^{20}\).

Molecular study

DNA extraction for antibiotic resistance gene molecular detection:

Total DNA was extracted from culture broth by pipetting 1.5 ml within Eppendorf tubes, centrifuged for five minutes at 4,300 x g, discarding the supernatant; 200 ul of TE buffer was then added, vortexed well, boiled for 10 minutes, and immediately placed on ice for 1 minute; the supernatant containing DNA was collected for use as DNA \(^{21}\). Antibiotic resistance genes (TEM, OXA, and GES) were detected using a PCR test with primers produced by (Macrogen/Korea) as in Table (1).

Table 1: The sequence of Primer that were used in the present study \(^{22}\).

<table>
<thead>
<tr>
<th>primer</th>
<th>DNA sequence (5′–3′)</th>
<th>Product Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bla-tem</td>
<td>F TCAACATTTCTGTGTGCCC</td>
<td>766</td>
</tr>
<tr>
<td></td>
<td>R AACTACGATACCGGGAGGGCT</td>
<td></td>
</tr>
<tr>
<td>Ges</td>
<td>F TCACTCTGCATATGCGTCGG</td>
<td>692</td>
</tr>
<tr>
<td></td>
<td>R ACTTGACCGACAGAGGCAAC</td>
<td></td>
</tr>
<tr>
<td>oxa</td>
<td>F AGATCCTTGACCCGCGAGTTG</td>
<td>928</td>
</tr>
<tr>
<td></td>
<td>R CGCCGTCCCATCGAAAAATC</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows the PCR thermal cycling condition. By electrophoresis in a 1.5 percent (w/v) agarose gel with 1 X TBE buffer and staining with SimpliSafe dye, the size of PCR products (5 L) was measured. A sizer100 bp DNA ladder (Intronbio/Korea) was used to determine the product size.

Table 2: PCR conditioned for amplification.

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>No. of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Initial Denaturation</td>
<td>95</td>
<td>(5) Minutes</td>
<td>1</td>
</tr>
<tr>
<td>2. Denaturation</td>
<td>95</td>
<td>(30) Second</td>
<td></td>
</tr>
<tr>
<td>3. Annealing</td>
<td>56 (TEM, OXA)</td>
<td>(45) Second</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>58 (GES)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Extension</td>
<td>72</td>
<td>(65) Second</td>
<td></td>
</tr>
<tr>
<td>5. Final extension</td>
<td>72</td>
<td>(5) Minutes</td>
<td>1</td>
</tr>
<tr>
<td>Storage</td>
<td>4</td>
<td>Hold</td>
<td></td>
</tr>
</tbody>
</table>

Condition of Polymerase Chain Reaction (PCR):

Conventional PCR was used to amplify a target DNA using specific primers. Three consecutive phases (denaturation, annealing, and elongation) of repeated cycles (amplicon) are often involved in PCR in order to produce a PCR product.

Ethical consideration:

The Al-Sader Medical Hospital's ethical committee gave its approval for this investigation [21/2/2024 – 7/27/584]
RESULT:

Isolate identification:

Between 100 isolates, Among the gram-negative bacteria were Escherichia coli (25%) and Klebsiella pneumonia (12%), among others, while the gram-positive bacteria comprised the aureus strain of Staphylococcus (12%) as shown in Table (3). The identification is done using the VITEK-2 compact system.

Molecular detection of antibiotic resistance genes:

The antibiotic resistance genes bla-TEM, GES, and bla-OXA were identified using characteristic band patterns on 1.5 percent Agarose. According to recent research, all isolates had their DNA amplified using specific primers for the Polymerase Chain Reaction (PCR). The PCR products for the bla-TEM gene (766 bp), GES gene (692 bp), and OXA gene (928 bp) were observed as indicated in Figure (A, B, C).

Table 3: Distribution of bacterial isolates of urinary tract infection

<table>
<thead>
<tr>
<th>Types of bacteria</th>
<th>Isolate</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>E. faecalis</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>K. pneumonia</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>E. aerogenes</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>C. freundii</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>P. mirabilis</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>K. oxytoca</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>S. typhi</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>A. baumanii</td>
<td>2%</td>
</tr>
<tr>
<td>Gram negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Image of gel electrophoresis with bands for bla-TEM gene at 766 bp]
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Figure: Electrophoresis of PCR results from extracted total DNA of bacterial isolate causing UTI using primers TEM, GES, and OXA on an ethidium bromide-stained agarose gel. The electrophoresis was done for 1.5 hours at 70 volts. lane (M), DNA molecular size marker (1500bp ladder).
A: Lanes (2- P. mirabilis, 3- K. pneumonia, 7- K. oxytoca, 8- E. coli, and 9- P. aeruginosa) all had positive TEM gene findings.
B: Lanes (1- E. aerogenes, 3- K. pneumonia, 6- C. freundii, 8- E. coli, and 9- P. aeruginosa) indicate GES gene positivity.
C: Lane (3- K. pneumonia, 6- C. freundii, 7- K. oxytoca, 8- E. coli, and 9- P. aeruginosa) has a positive OXA result.

DISCUSSION:

Isolate identification:
The study findings are consistent with those published by Shrestha et al. in 2016, who found Proteus species, K. pneumoniae, K. oxytoca, and E. coli., C. freundii, Pseudomonas spp., Enterobacter spp., and Acinetobacter spp. in UTIs (23).
E. coli was the most prevalent organism linked with UTI in this research, with K. pneumoniae coming in second, which is consistent with many previous investigations. E. coli is the most prevalent pathogen, regardless of age, gender, community, or nation. Although Gram-negative bacteria cause the majority of UTIs, Gram-positive bacteria have become a prominent cause in recent years. S. aureus was the third most frequent organism recovered, accounting for 12% of UTI cases (24-26). Other research (27) has shown similar results.

Molecular detection of antibiotic resistance genes:

(bla-TEM, GES, and OXA) genes were shown to be positive in the molecular identification of antibiotic resistance genes. This conclusion is consistent with that of (28), who discovered three resistance genes in E. coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa (29), discovered TEM in Klebsiella oxytoca and Proteus mirabilis. In another study done in Saudi Arabia, OXA was identified in Klebsiella oxytoca, Citrobacter freundii, and Acinetobacter baumanii (30), and GES was detected in Enterobacter aerogenes (31), and Citrobacter freundii (32). These enzymes are plasmid-encoding B-lactamases, which are widely found in K. pneumoniae and E. coli. TEM (temoniera), OXA (oxacillinase), and GES were found in additional treatment isolates of P. aeruginosa and Enterobacteriaceae. Other bacterial species have been found to have it (33).

A conflict of interest:

Not having any competing interests.

REFERENCES:


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الكشف الجزيئي لجينات مقاومة المضادات الحيوية

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تستلزم مقدمة الدراسة الاختبارات الإحصائية بواسطة نموذج VITEK-2 للعسلات البكتيرية في سلسلة اختبار بلينكلي(Columbia Phylogenetic) في مسافات الأمراض البولية في معيار 0.94 % . 

الكشف الجزيئي لجينات مقاومة المضادات الحيوية

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ان التهاب المسالك البولية هو من بين الأكثر شيوعًا بين الأمراض البولية، وتشمل البكتيريا المسببة في تهاب المسالك البولية P. aeruginosa، S. aureus، S. epidermidis، Enterobacter aerogenes، K. oxytoca، Citrobacter freundii، K. pneumonia، K. oxytoca، K. pneumonia، S. aureus، S. epidermidis، E. coli، P. mirabilis، K. oxytoca، P. aeruginosa، E. coli، K. pneumonia، K. oxytoca، Citrobacter freundii، S. aureus، S. epidermidis، E. coli، P. mirabilis، K. oxytoca.