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

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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. pneumonia *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. pneumonia*,* 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 microbial 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

is both extensively and multidrug resistant (MDR, XDR-MTB), as well as *methicillin-resistant Mycobacterium tuberculosis*, are 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 (sulfhydryl-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 integron-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 ℃ 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].

primer		DNA sequence (5′–3′)	Product Size (bp)
Bla-tem	F	TCAACATTTTCGTGTCGCCC	766
	R	AACTACGATACGGGAGGGCT	
Ges	F	TCACTCTGCATATGCGTCGG	692
	R	ACTTGACCGACAGAGGCAAC	
oxa	F	AGATCCTTGACCCGCAGTTG	928
	R	CGCCGTCCCATCGAAAAATC	

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.

Step	Temperature (°C)	Time	No. of cycles
1. Initial Denaturation	95	(5) Minutes	1
2. Denaturation	95	(30) Second	35
3. Annealing	56 (*TEM*, *OXA*) 58 (*GES*)	(45) Second	
4. Extension	72	(65) Second	
5. Final extension	72	(5) Minutes	1
Storage	4	Hold	

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:

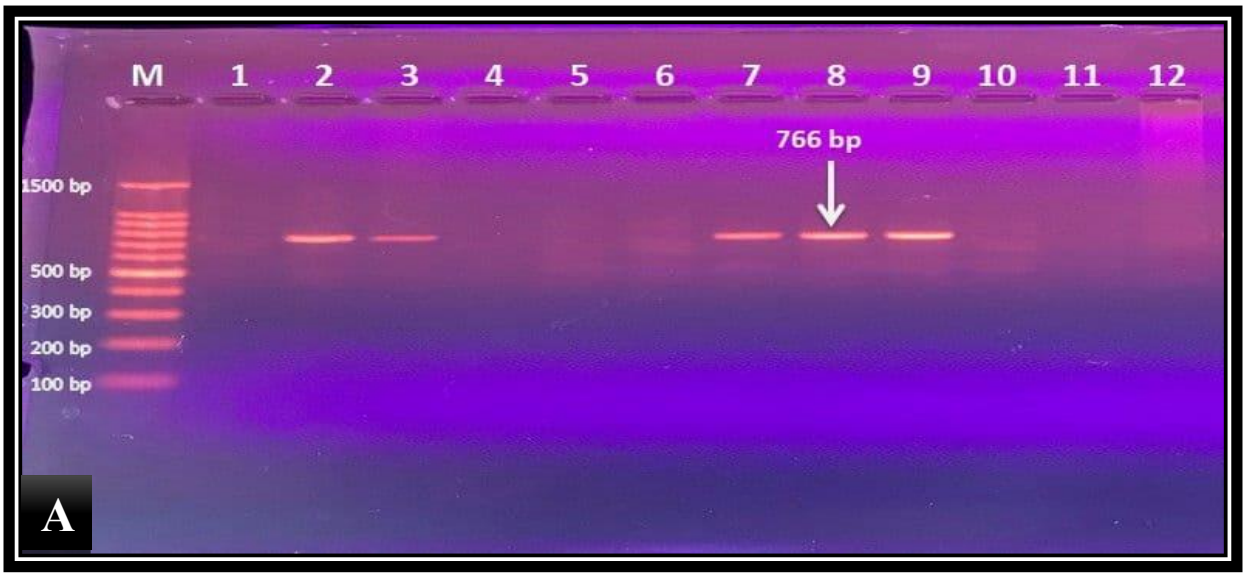
Between 100 isolates, Among the gram-negative bacteria were Escherichia coli (25%) and *Klebsiella pneumonia* (12%), among others, while the gram-positive bacteria comprised the aureus strain of Staphylococcus (12%) as shown in Table (3). The identification is done using the VITEK-2 compact system.

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

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



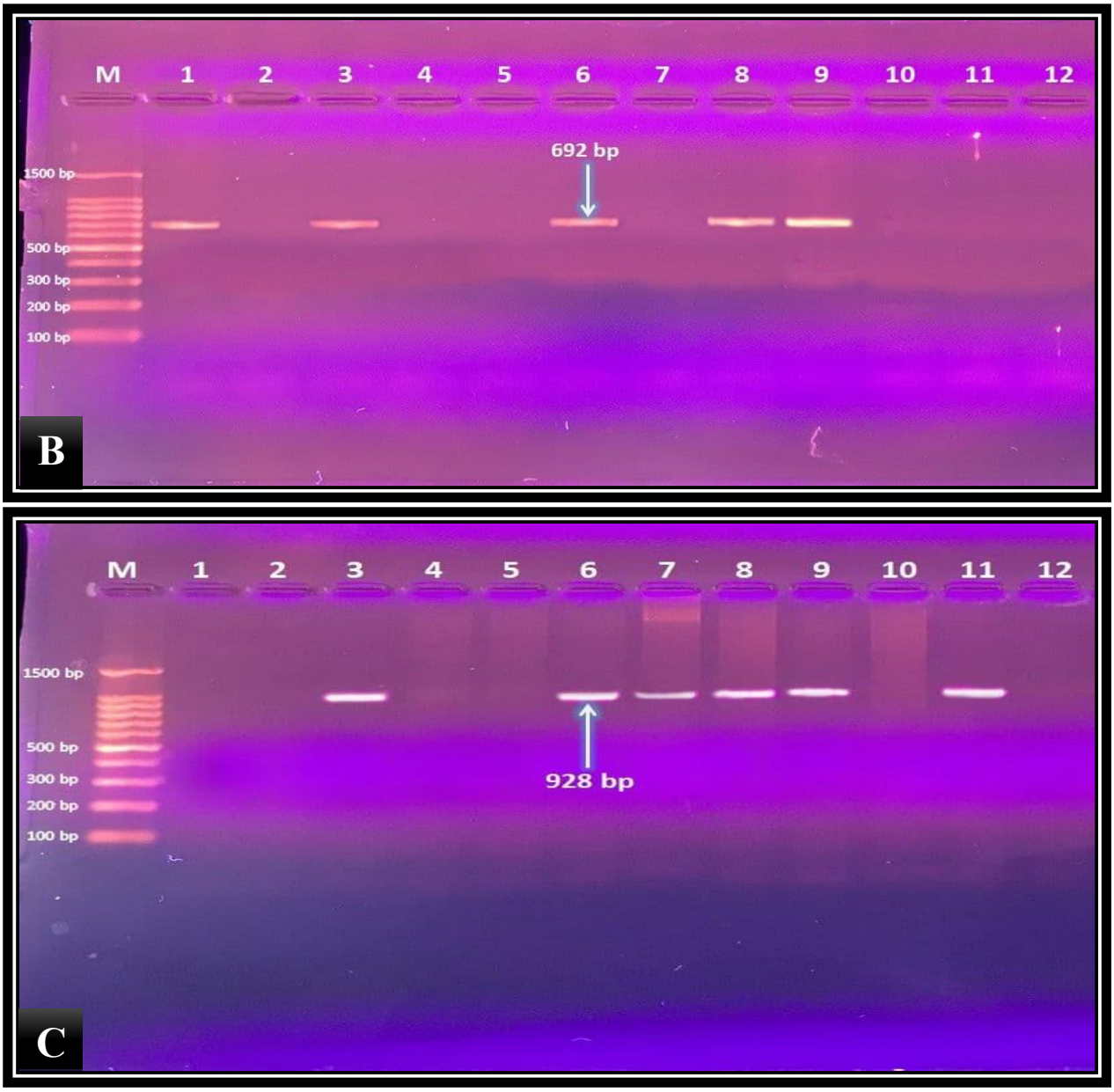


Figure: Electrophoresis of PCR results from extracted total DNA of bacterial isolate causing UTI using primers TEM, GES, and OXA on an ethidium bromide-stained agarose gel. The electrophoresis was done for 1.5 hours at 70 volts. lane (M), DNA molecular size marker (1500bp ladder).
A: Lanes (2- ***P. 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. fruendi, 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.

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الكشف الجزيئي لجينات مقاومة المضادات الحيوية (GES,OXA ,TEM) في مسببات الامراض البولية

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قسم تقنيات المختبرات الطبية - المعهد التقني - جامعة الفرات الاوسط التقنية – كوفة – العراق

تعتبر مقاومة المضادات الحيوية واحدة من أخطر التهديدات التي تهدد الصحة العامة على مستوى العالم؛ يمكن أن يقتل 700 ألف شخص ويمكن أن يرتفع إلى عشرة ملايين بحلول عام 2050 لذا تتضمن الدراسة الكشف عن البكتريا الحاوية على الجينات المقاومة للمضادات الحيوية، وتعد عدوى المسالك البولية من الالتهابات السائدة بين الناس والتي تنتج عن البكتيريا المسببة للأمراض. في أكثر من 80٪ من الحالات، يحدث التهاب المسالك البولية بسبب بكتيريا، والكائن الأكثر شيوعًا المسؤول عن هذه البكتيريا هو الإشريكية القولونية (E. coli) التي تعد جزءًا من النباتات الطبيعية في الأمعاء، والبكتيريا سالبة الجرام الأكثر شيوعًا في الدراسة هي E. القولونية حوالي 25%، K. الالتهاب الرئوي حوالي 12%، الأمعائية aerogenes حوالي 10% وغيرها في حين أن البكتيريا إيجابية الجرام الأكثر شيوعا التي تسبب التهاب المسالك البولية هي S. aureus 12%، S. epidermidis حوالي 8% والمكورات المعوية البرازية حوالي 4%. . تم تشخيص العزلات البكتيرية باستخدام النظام المورفولوجي والكيميائي الحيوي ونظام VITEK-2 المدمج. تتضمن الدراسة الكشف عن المقاومة للمضادات الحيوية بنظام VITEK-2 المضغوط تتضمن الدراسة التركيز على الجين المقاوم المضاد الحيوي للكشف عن البكتيريا التي تحتوي على الجين الأكثر مقاومة للمضادات الحيوية ويؤدي في النهاية إلى تعديلها في المستقبل. الجين المستخدم في الدراسة هو (TEM بنسبة 41%، GES بنسبة 41%، وOXA بنسبة 50%)، الجينات المقاومة الثلاثة الموجودة في K. pneumonia وE. coli وP. aeruginosa لكن البكتيريا الأخرى تحتوي أيضًا على واحد من الجينات التي تم اختبارها مثل TEM الموجودة في K. oxytoca وP.mirabilis، وGES في Enterobacter aerogenes وCitrobacter freundii، في حين أن جين OXA الموجود في A. baumanii، وC.freundii، وK. oxytoca.