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Research Article

Antibiotic resistance gene among Carbapenem-Resistant Gram-negative Bacteria Harbour NDM gene in Al-Najaf City, Iraq

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Abstract

The spread of carbapenem-resistant bacteria has increased across many species and genera. As a result, the effectiveness of carbapenem treatment has diminished over time. Therefore, the aim of this work was to study carbapenem-resistant Gram-negative bacterial isolates. The current study were revealed out of 1031 clinical samples from different sources, The distribution of total patients by age group including. The age group was (1-20 years) the number of patients was 222(21.53%), the number of females was 141(19.05%)and while the males were 81(27.83%). For the age group (21-40 years) number of patients was 264(25.60%), number of females was 188(25.40%) and the number of males was 76(26.11%). The age group of (41-60 years) was the number of patients 435(42.19%) number of females was 355(47.97%) and the number of males 80(27.49%). Over 61 the number of patient 110(10.66%), the number of females was 56(7.56%) and the number of males was 54(18.55%). The current study showed that females are more likely to be affected by drug resistance than males in the age group of (41-60). By using PCR technique to screen genes responsible for carbapenem resistance. Molecular data from this work showed revealed high rate and frequencies of *NDM-3*, *Tem*, *Tet A* and *Sul -1* genes which reached 28/30(93.33%) respectively. While *Tet B*, *Qnr B*, *Sul -2* and *Sul-3* were 29/30(96.61%), 27/30(90%), 23/30(76.66% and 5/30(16.66%) respectively.

1. Introduction

Drug resistance is a significant and escalating public health concern that undermines the effectiveness of antimicrobial therapies, leading to increased morbidity and mortality rates [1]. This phenomenon occurs when pathogens, including bacteria, viruses, fungi, and parasites, evolve mechanisms to withstand the effects of medications that once successfully eradicated them [2]. The emergence of drug-resistant strains is primarily driven by factors such as the overuse and misuse of antibiotics, inadequate infection control practices, and the lack of new drug development [3]. Carbapenems were the last β-lactams retaining near-universal anti-Gram-negative activity [4]. Carbapenems, most notably imipenem and meropenem, have been recognized as the most potent β-lactams against multidrug-resistant Gram-negative bacteria because of their high affinity for penicillin-binding proteins, their stability against extended-spectrum β-lactamases (ESBLs) and their permeability of the bacterial outer membranes [5]. This carbapenemase has also been identified in *P. aeruginosa* and *Acinetobacte*r spp [6]. *Acinetobacter* sp. may play a pivotal role for spreading genes for its natural reservoir to *Enterobacteriaceae*, [7]. Such as *(NDM*) is a type of metallo-β-lactamase (MBL) able to hydrolyze most β-lactams (including carbapenems) but not monobactams [8]. It has poor activity against amdinocillin, an extended-spectrum penicillin antibiotic of the aminopenicillin family [9]. Carbapenemases are members of the β-lactamases divided into classes A, B, and D based on their molecular structures [10]. New Delhi metallo-β-lactamase *(NDM*) enzymes are the latest carbapenemases to be recognized and since 2008 have been reported worldwide, *NDM*-positive strains have been

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identified worldwide, with the highest prevalence in the Indian subcontinent, the Middle East, and the Balkans [4]. Most *blaNDM*-carrying plasmids belong to limited replicon types (IncX3, IncFII, or IncC) [11]. Commonly used phenotypic tests cannot specifically identify *NDM* [12]. Lateral flow immunoassays specifically detect *NDM*, and molecular approaches remain the reference methods for detecting *blaNDM* [13]. Polymyxins combined with other agents remain the mainstream options of antimicrobial treatment. Compounds able to inhibit *NDM* have been found, but none have been approved for clinical use. Outbreaks caused by *NDM*-positive strains have been reported worldwide, attributable to sources such as contaminated devices [14]. Evidence-based guidelines on preventing and controlling carbapenem-resistant Gram-negative bacteria are available, although none are specific for *NDM*-positive strains. *NDM* will remain a severe challenge in healthcare settings, and more studies on appropriate countermeasures are required. *NDM* enzymes are composed of 270 amino acids, containing two zinc ions at the active site, where the hydrolysis of β-lactams takes place [15]. The secondary structure of *NDM* enzymes contains 9 -helices, 17 β-strands, and 3 turns [16]. *NDM* carbapenemases are a rapidly emerging and troublesome family of β-lactamases [17]. *NDM-1* was first identified in a *Klebsiella pneumonia* strain isolated from a Swedish patient who had been hospitalized in New Delhi, India in 2008 [18].

2. Methods

2.1. Ethical approval

Every the sample of utilized in this study was obtained with the consent and authorization of the patients and healthcare institutions .

2.2. Patients and Specimens Processing

The current research included a total of 1031 individuals gathered from various clinical sources, selected randomly,the samples were blood, sputum, burn wound swab, ear swab, throat swab and urine took place in main Al-Najaf City were involved in the diagnostic process. This medical intervention spanned several months, from February 2024. Each specimen was streaked on blood agar, and macConkey agar and incubated aerobically at 37°C overnight in a sterile environment [19].

2.3. Carbapenem agents susceptibility of Gram-negative bacteria

The Kirby-Bauer method was used to test the antibacterial medicines imipenim, doripenem and meropenem on all gram-negative bacteria isolated in this work. The testing was done on Mueller-Hinton agar [20]. To prepare the inoculation of all isolates, the overnight growth of each tested isolate was suspended in sterile normal saline that had been adjusted to a 0.5 McFarland standard tube. An analysis was conducted on antibacterial discs available for purchase at retail establishments. The zone diameters were interpreted following the directions given in the Clinical Laboratory Standards Institute (CLSI) guidance [20].

2.4. Identification among carbapenem-resistant Gram-negative bacteria

All carbapenem-resistant gram-negative bacteria obtained in current work were streaking on MacConkey agar to obtain single colony for each bacteria, then the identified was done based on microscopic, morphological, oxidase, and motility features, as well as certain important biochemical assays. The ultimate identification was carried out with the automated Vitek-2 compact system employing ID-GN card [21].

2.5. Molecular investigation of antibiotic resistance genes among carbapenem-resistant Gram-negative bacteria

Using the guidelines from the manufacturing firm, we used a genomic DNA extraction micro kit (Favorgen, South Korea) to gather all the nucleic acids from all carbapenem-resistant Gram-negative bacteria. This was executed in compliance with the manufacturer's methodology. After confirming the integrity of the whole DNA sample by putting it in a deep freezer set to -20 degrees Celsius, Firstly screening about *NDM-3* gene by specific primer using monoplex PCR, then only carbapenem-resistant Gram-negative harbored *NDM-3* gene choosing to a PCR analysis was conducted to examine the genes indicated in Table 1. The gel documentation equipment was used to analyses the migration of PCR amplification bands on a 1% agarose gel. Subsequently, the bands were stained with safe stain at a concentration of 0.5 g/ml [21]. Positive products of *NDM-3* gene were sent to Macrogen company (Korea) to obtain nucleic acid sequencing of *NDM-3* gene, and then compare data with international strain using NCBI web.

2.6. Statistical analysis

The findings were analysed and computed based on numerical data and percentages utilising computer tools [29].

3. Results

3.1. Patients and Specimens Collection

During the period From February 2024 to May 2025. A total of 1031 clinical specimens from non-duplicate patients were randomly collected and screened for carbapenem-resistant isolates. The distribution of total patients by age group including the age group was (1-20 years) the number of patients was 222(21.53%), the number of females was 141(19.05%) and while the males were 81(27.83%). For the age group (21-40 years) number of patients was 264(25.60%), number of females was 188(25.40%) and the number of males was 76(26.11%). The age group of (41-60 years) was the number of patients 435(42.19%) number of females was 355(47.97%) and the number of males 80(27.49%). Over 61 the number of patient 110(10.66%), the number of females was 56(7.56%) and the number of males was 54(18.55%). The data is in Table 2.

Table 1: Primer Sequence and condition.

Target	Primer target	Sequence of primer 5' to 3'	Size of Product (bp)	Reference
NDM-3	F R	CCTGGACCAATGACCAGACC AATACCTTGAGCGGGCCAAA	274bp	Tada [22]
QnrB	F R	ATGACGCCATTACTGTATAA GATCGCAATGTGTGAAGTTT	560 bp	Jacoby [23]
Tem	F R	CAGCGGTAAGATCCTTGAGA ACTCCCCGTCGTGTAGATA	643bp	Ensor [24]
Tet A	F R	TTGGCATTCTGCATTCACTC ATCCAGATCCGCAAAGGTTA	494bp	Guardabassi [25]
Tet B	F	CAGTGCTGTTGTGTCATTA A	571 bp	Call [26]
Sul-1	F R	GTGACGGTGTTCGGCATTCT TCCGAGAAGGTGATTGCGCT	921 bp	Tamocha [27]
Sul-2	F R	CGGCATCGTCAACATAACCT TGTGCGGATGAAGTCAGCTC	721 bp	Tamocha [27]
Sul-3	F R	CAGATAAGGCAATTGAGCATGCTCTGC GATTTCCGTGACACTGCAATCATT	569 bp	El-Kazzaz [28]

Table 2: Distribution of total patients according to age groups.

Age group	Years patients No (%)	Female patients No (%)	Male patients No (%)
1-20	222(21.53%)	141(19.05%)	81(27.83%)
21-40	264(25.60%)	188(25.40%)	76(26.11%)
41-60	435(42.19%)	355(34.43%)	80(27.49%)
Over 61	110(10.66%)	56(7.56%)	54(18.55%)
Total	1031(100%)	740(71.77%)	291(28.22%)

Table 3: Susceptibility of Gram negative bacteria to carbapenem by vitek-2 techniques.

Total gram negative	Carbapenem resistant	Intermedite	Sensitive
477 (73.49%)	114(23.89%)	25 (5.24%)	338 (70.85%)

Antibiotic susceptibility of *Acinetobacter baumannii, E. coli, Klebsiella pneumoniae, Pseudomonas aeroginosa, Enterobacter clocae, Serrtia marcesens and Burkholderia. cepcia* Eigh isolates of *Acinetobacter baumannii* were absolutely resistant to meropenem 8(100%) and eight isolates of *Acinetobacter baumannii* showed absolute resistance against doripenem 8(100%), while impenem showed resistance to 5(62.5%) and Intermedet 3(37.5%). eight isolates of *Esherichia colli* were absolutely resistant to impenem 8(100%), while seven isolates of. *Esherichia colli* were resistant to doripenem 7(87.5%) and One isolate showed moderate resistance to doripenem 1(12.5), also seven isolates of. *Esherichia colli* were resistant to meropenem 7 (87.5%), One isolate showed moderate resistance to meropenem 1(12.5). Six isolates of *Klebsiella pneumonia* absolutely resistant to impenem 6(100%), absolutely resistant to meropenem 6(100%) and doripenem 6(100%). Two isolates of *Enterobacter* clocae absolutely showed absolutely resistant to impenem 2(100%), meropenem 2(100%) and doripenem 2(100%). One isolates of *Serrtia* absolutely resistant to impenem 1(100%), meropenem 1(100%) and doripenem 1(100%). Two isolates of *psedomonas aeruginosa* showed impenem resistance 2(66.66%), while one isolate of pseudomonas appear sensitive to impenem 1(33.33%), two isolates resistance to doripenem 2(66.66%) and one isolate intermediate 1(33.33%), Two isolates of *psedomonas aeruginosa* showed meropenem resistance 2(66.66%), while one isolate of *pseudomonas* appear sensitive to meropenem 1(33.33%). Two isolates of *Burkholderia cepcia* absolutely resistant to meropenem 2(66.66%).

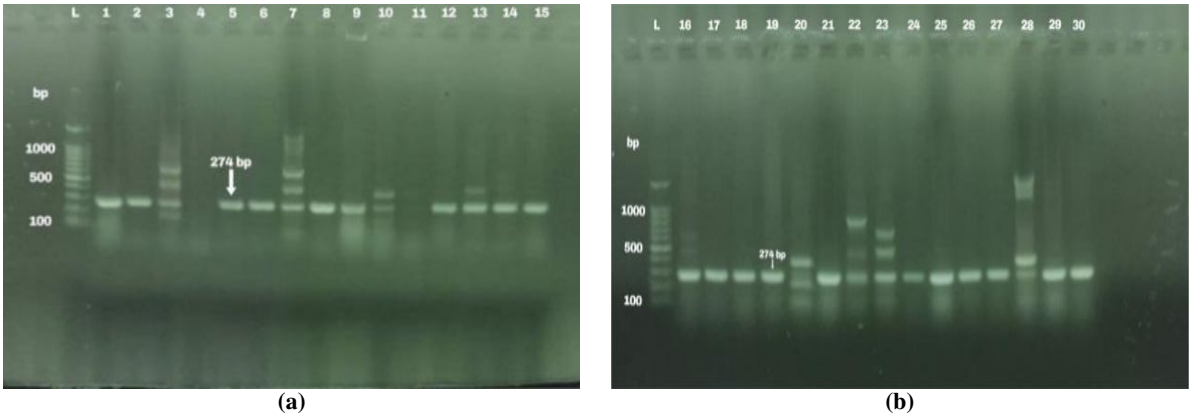
3.2. Molecular study of *NDM-3, Tem, Tet A, Sul-1, Sul-2, Sul-3Tet B, and Qnr B genes* in Clinical Isolates of Gram negative bacteria

PCR results showed high frequencies of NDM-3, *Tem*, *Tet A* and *Sul-1* genes which reached 28/30 (93.33%), in Fig 1, 2, 3 and 4 respectively. While *Tet B*, *Qnr B*, *Sul-2* and *Sul-3* were 29/30(96.61%), 27/30 (90%), 23/30 (76.66%) and 5/30 (16.66%) respectively, in Fig 5, 6, 7 and 8 respectively.

4. Discussion

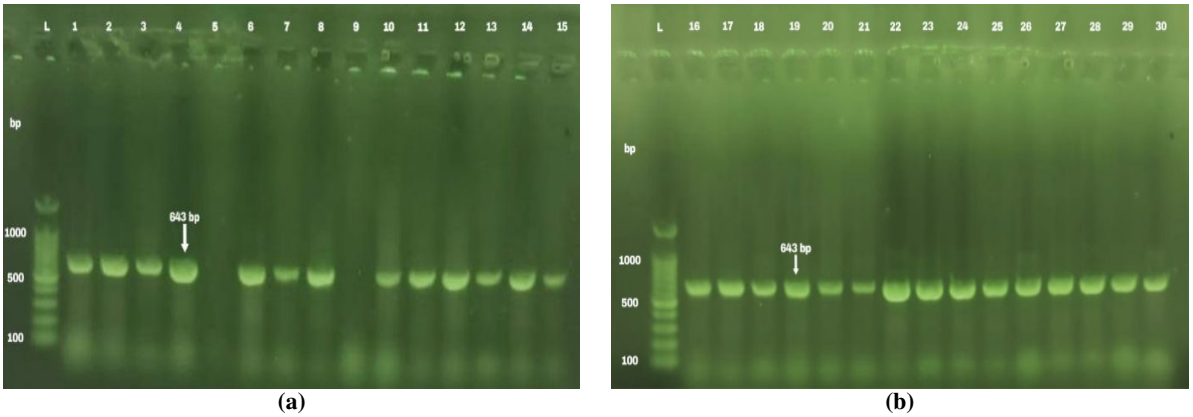
The results of the current study indicate that the percentage of women aged 41-60 is higher than that of men in terms of susceptibility to drug resistanc. The results of the current study are similar to age groups in other local and global studies, including : The data from this study is consistent with the results of a study conducted in Erbil Governorate / Iraq, which confirms that the infection rate among females is higher than that among males. Only 57 bacteremia isolates were isolated from 1023 samples from (2015-2021), in our study the percentage of females infected with bacteremia were more than the males, females being 29/1023 (2.84%) and males being 28/1023 (2.74%) (Ali et al ., 2022). While global studiesfrequency of isolation with the age-group of the patient, significant proportion of the isolates were recovered from patient within the age- group 20-29 years (25.6%), followed by <10years (22.0%) and 30-39 years (20.5%). *Escherichia coli* predominate in all the age-groups except in <10 and 40-49 years in which *P. aeruginosa* accounted 6.5% in <10 years, and *Klebsiella spp* 3.4% in 40-49 years respectively.

The results of the current study similar from the results of the study of Iraq. The study isolates showed highest antimicrobial resistance to



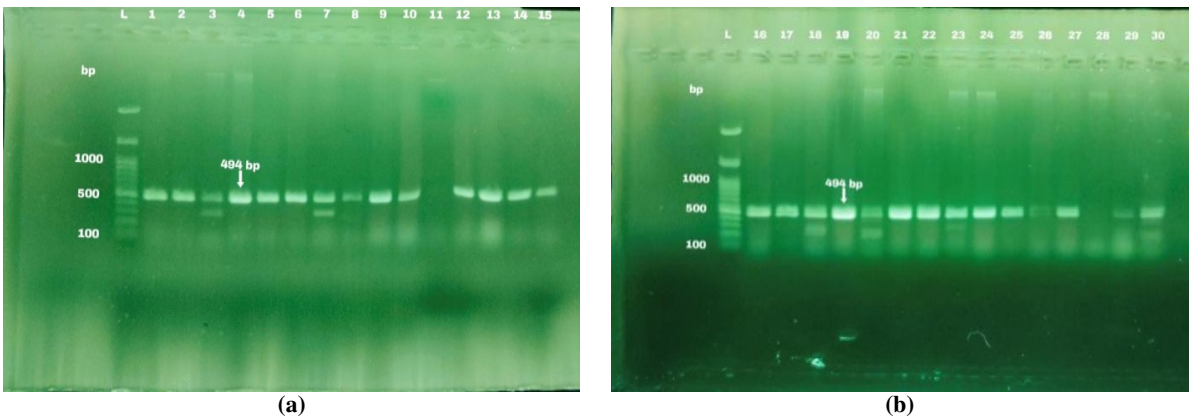
(a) **(b)**

Figure 1: PCR products for *NDM-3* gene of *Acinetobacter bumannii, Esherichia coli, Klabsielae pneumonia,pseudomonas aeruginosa, Enterobacter cloacae, Serrtia marscence and Burkholderia cepcia*



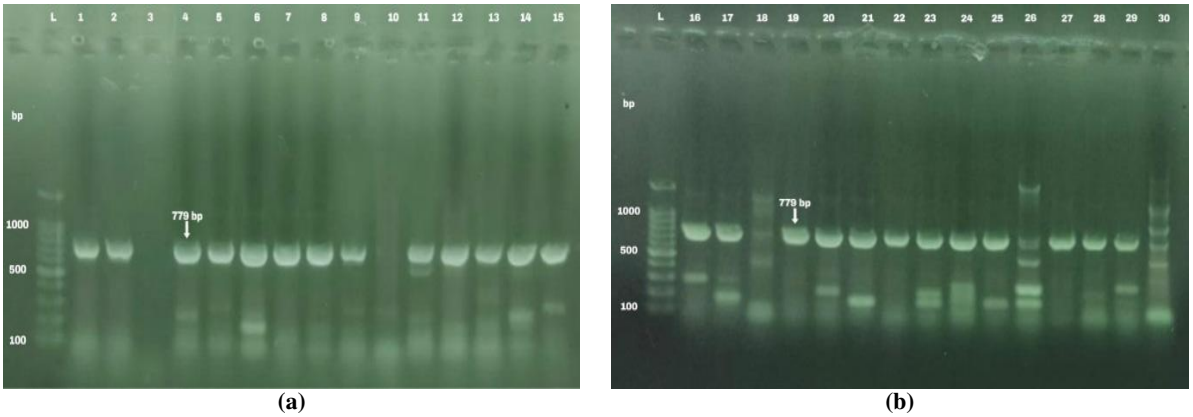
(a) **(b)**

Figure 2: PCR products for *Tem gene* of *Acinetobacter bumannii, Esherichia coli, Klabsielae pneumonia,pseudomonas aeruginosa, Enterobacter cloacae, Serrtia marscence and Burkholderia cepcia*



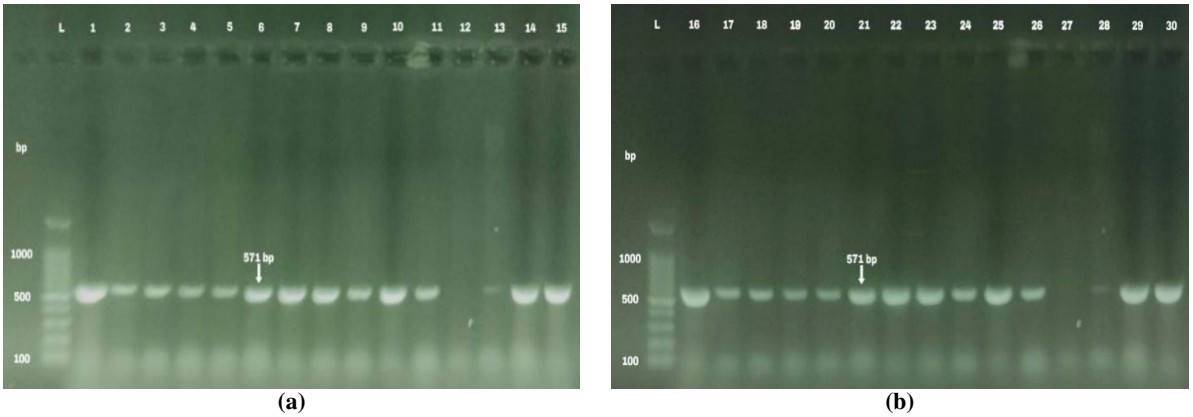
(a) **(b)**

Figure 3: PCR products for *Tet-A* gene of *Acinetobacter bumannii, Esherichia coli, Klabsielae pneumonia,pseudomonas aeruginosa, Enterobacter cloacae, Serrtia marscence and Burkholderia cepcia*



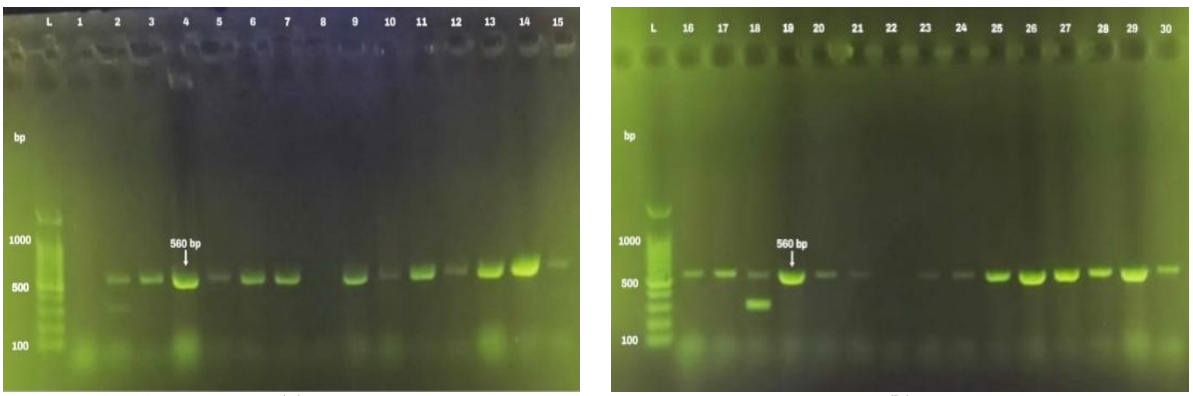
(a) **(b)**

Figure 4: PCR products for *Sul-1* gene of *Acinetobacter bumannii, Esherichia coli, Klabsielae pneumonia,pseudomonas aeruginosa, Enterobacter cloacae, Serrtia marscence and Burkholderia cepcia*



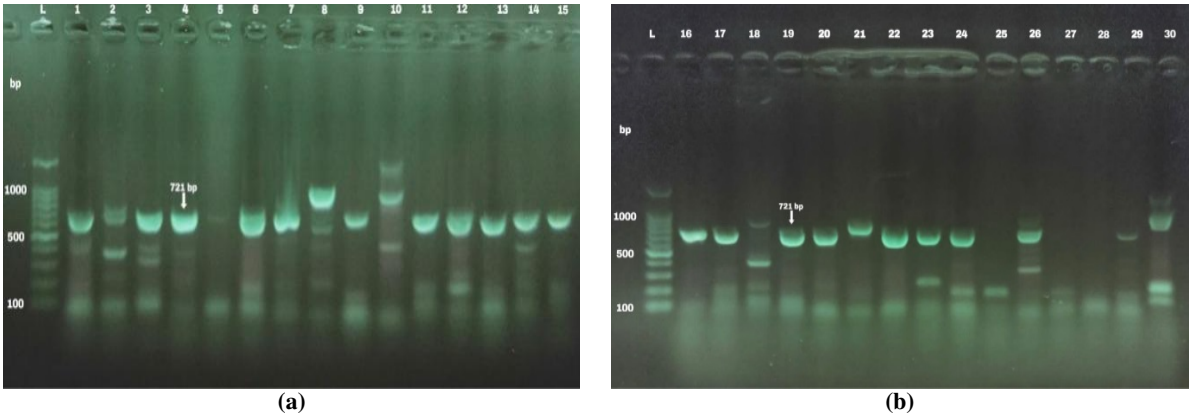
(a) **(b)**

Figure 5: PCR products for *Tet-B* gene of *Acinetobacter bumannii, Esherichia coli, Klabsielae pneumonia, pseudomonas aeruginosa, Enterobacter cloacae, Serrtia marscence and Burkholderia cepcia*



(a) **(b)**

Figure 6: PCR products for *Qnr – B gene* gene of *Acinetobacter bumannii, Esherichia coli, Klabsielae pneumonia, pseudomonas aeruginosa, Enterobacter cloacae, Serrtia marscence and Burkholderia cepcia*



(a) (b)

Figure 7: PCR products for *Sul-2* gene of *Acinetobacter bumannii, Esherichia coli , Klabsielae pneumonia, pseudomonas aeruginosa, Enterobacter cloacae, Serrtia marscence and Burkholderia cepcia*

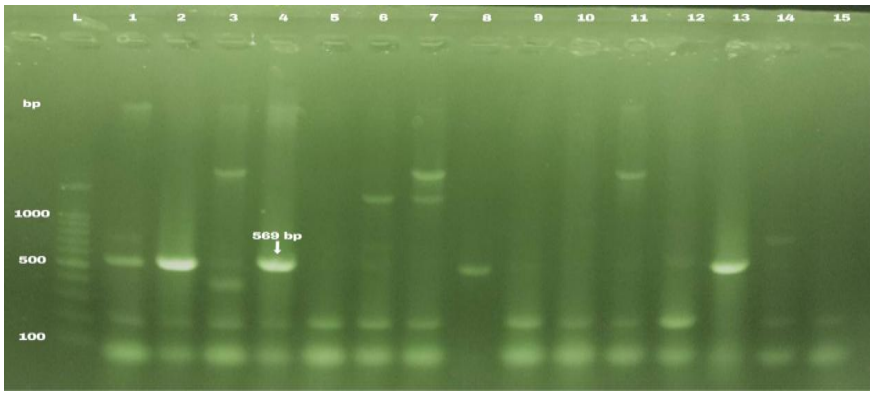


Figure 8: PCR products for *Sul-3* gene of *Acinetobacter bumannii, Esherichia coli, Klabsielae pneumonia, pseudomonas aeruginosa, Enterobacter cloacae, Serrtia marscence and Burkholderia cepcia*

piperacillin, cefepime, ceftriaxone (100%), ceftazidime (97.3%), and lowest antimicrobial resistance to imipenem (36%) (Al-Ouqaili et al ., 2024). The results of the current study differ from the results of the study of Lebanese. 4 patients with documented *B. cepacia* infections isolated from sites other than the respiratory tract admitted , Most of the isolated *B. cepacia* were susceptible to ceftazidime, carbapenems.

Also Aglobal study in Nigeria total of 150 swab samples were collected from fomites, health care workers, and catheters of patients suffering from urinary tract infection (UTI), this study demonstrated bla_{CTX-M} and bla_{TEM} was detected in 76.5% and 17.6% of the isolates respectively [30]. Molecular assay of the this work pointed that all 16 (100%) of *B. cepacia* isolates carried *bla-TEM* and *bla-CTX-M* while 15(93%) harbored *bla-OXA* gene, At same respect, 8(50%) and 4(25%) of isolates were carried the *bla-CTX-M-15* and *bla-SHV-12* respectively [31]. Global study in waste water effluents. Tetracycline and beta-lactam resistance markers were found; 70% and 92% of the isolates possessed *tetA* and *ampC* genes. The isolates showed high level of resistance to antibiotics [32]. Local study in Thi-Qar, Iraq, of *E. coli* isolates the *sul-1* gene was found only in 56.67% [33]. City in Baghdad (National Center for Educational Laboratories , the *Sul-1* gene prevalence were 11(31%), Moreover, the resisted isolates with Sul2 gene prevalence was 8(23%) [34]. Global study In 200 sulfonamide-resistant Portuguese Salmonella isolates, 152 sul1, 74 sul2, and 14 sul3 genes were detected [35].

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