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Molecular and Phenotypic Detection of Carbapenemase-Encoding Genes among Bacterial Isolates from Various Clinical Infections

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Abstract

This study was conducted to perform phenotypic and molecular detection of carbapenems-producing bacteria isolated from various infections. A total of 140 clinical samples were collected from patients of both sexes and various ages. The samples were collected from various hospitals in Salah al-Din Governorate, during the period from August 4, 2025, to December 10, 2025. The results showed that 103 samples (73.6%) exhibited bacterial growth on different culture media. The infection rates by sample type were as follows: urine, with 40 samples (28.6%); respiratory tract 6 samples (4.3%); blood, 3 samples (2.1%); wounds, 8 samples (5.7%); burns, 25 samples (17.9%); stool, 10 samples (7.1%); semen, 10 samples (7.1%); vaginal, 20 samples (14.3%); catheter, 10 samples (7.1%); and bodily fluids, 8 samples (5.7%). Bacterial isolates were identified by manual methods and Vitek 2 system, and the results revealed 24 types of bacterial species with different rates. , with *Escherichia coli* being the most prevalent at 27.18%, followed by *Staphylococcus aureus* at 18.45%. *Klebsiella pneumoniae* accounted for 6.80%, while *Staphylococcus epidermidis* and *Streptococcus agalactiae* each accounted for 4.85%. Other isolates included *Acinetobacter baumanii., Citrobacter spp., Stenotrophomonas maltophilia and Pseudomonas aeruginosa*, each at 3.88%, followed by *Enterobacter cloacae*, *Streptococcus pneumoniae*, each at 2.91%. Additionally, *Burkholderia cepacia, Proteus vulgaris, Streptococcus pyogenes* and *Kocuria kristinae*, *were* isolated at 1.94% each. Finally, 10 species were isolated at 0.97% each, including *Klebsiella oxytoca, Pantoea spp., Streptococcus mutans, Streptococcus viridans, Enterococcus casseliflavus*, *Staphylococcus intermedius, Staphylococcus auricularis, Staphylococcus capitis*, and *Staphylococcus lentus*. The results of The distribution of carbapenem-resistant bacteria was as follows: *Escherichia coli* accounted for 28 isolates, of which 5 (17.9%) were carbapenem-resistant; *Klebsiella pneumoniae* accounted for 7 isolates, of which 6 (85.7%) were resistant; *Pseudomonas aeruginosa* accounted for 4 isolates, all of which were resistant; *Acinetobacter baumannii* accounted for 4 isolates, all resistant; and *Stenotrophomonas* accounted for 4 isolates, all resistant. The results of molecular detection revealed the presence of specific resistance genes in each bacterial species. *E. coli* harbored the *blaOXA-1* gene in 88% of isolates, *K. pneumoniae* harbored the *blaKPC* gene in 66.6% of isolates, *A. baumanii* harbored the *blaOXA-48* gene in 50% of isolates, while *P. aeruginosa* (*blaVIM*-type) and S. *maltophilia* (*blaL1*) did not harbor the gene.

Keywords: Carbepenem, Resistance, phenotype, Genotypem Bacteria

الكشف الجزيئي والمظهري عن الجينات المشفرة للكاربابينيميز بين العزلات البكتيرية من مختلف أنواع العدوى السريرية

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الخلاصة

اجريت هذه الدراسة بهدف الكشف الظاهري والجزيئي عن البكتيريا المنتجة للكاربابينيم والمعزولة من حالات عدوى مختلفة، حيث تم جمع ما مجموعه 140 عينة سريرية من مواقع مختلفة من الجسم (البول، الجهاز التنفسي، الدم، الجروح، الحروق، البراز، السائل المنوي، المهبل، القسطرة، سوائل الجسم) من المرضى الداخليين والخارجيين والمرضى المقيمين من كلا الجنسين ومختلف الأعمار. تم جمع العينات من مستشفيات مختلفة في محافظة صلاح الدين، شملت مستشفى سامراء العام، مستشفى تكريت التعليمي، ومستشفى العلم العام، وذلك خلال الفترة من 4 أغسطس 2025 إلى 10 ديسمبر 2025 .أظهرت النتائج أن 103 عينات (بنسبة 73.6%) أظهرت نموًا بكتيريًا على مختلف الأوساط الزرعية. وجاءت معدلات العدوى حسب نوع العينة كالتالي: البول 40 عينة (28.6%)، الجهاز التنفسي 6 عينات (4.3%)، الدم 3 عينات (2.1%)، الجروح 8 عينات (5.7%)، الحروق 25 عينة (17.9%)، البراز 10 عينات (7.1%)، السائل المنوي 10 عينات (7.1%)، المهبل 20 عينة (14.3%)، القسطرة 10 عينات (7.1%)، وسوائل الجسم 8 عينات.(5.7%) ،تم التعرف على العزلات البكتيرية باستخدام الطرق اليدوية ونظام Vitek 2 ، وأظهرت النتائج وجود 24 نوعًا بكتيريًا، حيث كانت الإشريكية القولونية (*Escherichia coli*) هي الأكثر انتشارًا بنسبة 27.18%، تليها المكورات العنقودية الذهبية (*Staphylococcus aureus*) بنسبة 18.45%. شكلت الكلبسيلا الرئوية (*Klebsiella pneumoniae*) بنسبة 6.80%، بينما شكلت كل من المكورات العنقودية البشروية (*Staphylococcus epidermidis*) والمكورات العقدية (*Streptococcus agalactiae*) بنسبة 4.85% لكل منهما. تضمنت العزلات الأخرى كلًا من الأسينيتوباكتر (*Acinetobacter spp.*)والسيروباكتر (*Citrobacter spp.*) والستينوتروفوموناس مالتوفيليا (*Stenotrophomonas maltophilia*) والزائفة الزنجارية (*Pseudomonas aeruginosa*) بنسبة 3.88% لكل منها، تليها كل من الإنتيروباكتر كلواكي (*Enterobacter*

(*cloacae*والمكورات العقدية الرئوية (*Streptococcus pneumoniae*) بنسبة 2.91% لكل منهما. بالإضافة إلى ذلك، تم عزل كل من البوركهولدرية سيباسيا (*Burkholderia cepacia*) والبروتيوس الشائع (*Proteus vulgaris*) والمكورات العقدية المقيحة (*Streptococcus pyogenes*)والكوكوريا كريستينية (*Kocuria kristinae*) بنسبة 1.94% لكل منها. وأخيرًا، تم عزل 10 أنواع بنسبة 0.97% لكل منها، شملت الكلبسيلا أوكسيتوكا (*Klebsiella oxytoca*) والبنتويا (*Pantoea spp.*) والمكورات العقدية الطافرة (*Streptococcus mutans*)والمكورات العقدية المخضرة (Streptococcus viridans) والإنتيروكوكوس كاسي فلافوس (*Enterococcus casseliflavus*)والمكورات العنقودية المتوسطة (*Staphylococcus intermedius*) والمكورات العنقودية الأذنية (*Staphylococcus auricularis*)والمكورات العنقودية الرأسية (*Staphylococcus capitis*) والمكورات العنقودية البطيئة (*Staphylococcus lentus*). تم إجراء اختبار الحساسية للمضادات الحيوية للعزلات البكتيرية باستخدام الطرق اليدوية ونظام Vitek 2 ضد عشرة مضادات حيوية (البنسلين، السيبروفلوكساسين، الليفوفلوكساسين، السيفترياكسون، النيتروفورانتوين، الفانكومايسين، حمض النالييديكسيك، الجنتاميسين، الميروبينيم، والإيميبينيم). أظهرت النتائج حساسية متباينة تجاه المضادات الحيوية المختبرة. وعند التركيز على مضادات الكاربابينيم (الإيميبينيم والميروبينيم)، تم اكتشاف مقاومة في 23 عزلة بكتيرية سالبة لصبغة غرام. جاء توزيع البكتيريا المقاومة للكاربابينيم على النحو التالي: الإشريكية القولونية (*E. coli*) شكلت 28 عزلة، منها 5 عزلات (بنسبة 17.9%) كانت مقاومة للكاربابينيم؛ الكلبسيلا الرئوية (*K. pneumoniae*)شكلت 7 عزلات، منها 6 عزلات (بنسبة 85.7%) مقاومة؛ الزائفة الزنجارية (*P. aeruginosa*) شكلت 4 عزلات، وجميعها مقاومة؛ الأسينيتوباكتر بوماني (*A. baumannii*) شكلت 4 عزلات، وجميعها مقاومة؛ والستينوتروفوموناس (*S. maltophilia*) شكلت 4 عزلات، وجميعها مقاومة. تم إجراء الكشف الجزيئي لجينات مقاومة الكاربابينيم باستخدام تفاعل البوليميراز المتسلسل (PCR) على العزلات الـ 23 السالبة لصبغة غرام. أظهرت النتائج وجود جينات مقاومة محددة في كل نوع بكتيري: احتوت *E. Coli* على جين blaOXA-1 في 88% من العزلات، بينما احتوت الكلبسيلا على جين blaKPC في 66.6% من العزلات، وأظهرت الأسينيتوباكتر وجود جين-blaOXA 48في 50% من العزلات في المقابل، لم تحتوي كل من الزائفة الزنجارية) التي فُحصت لجين (blaVIM-type والستينوتروفوموناس) التي فُحصت لجين (blaL1 على هذه الجينات .

الكلمات المفتاحية : الكاربابنيم، المقاومة، النمط الظاهري، النمط الجيني للبكتيريا

1. Introduction

Antibiotic resistance in bacteria is one of the biggest global health challenges of the modern era. It leads to a reduction in the effectiveness of available medical treatments and an increase in the rates of drug-resistant bacterial infections, Carbapenem antibiotics are used as a primary treatment option to combat infections caused by multi-drug-resistant bacteria, including *Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacteriaceae,* among other [1]. Given their clinical importance, the emergence and spread of carbapenem resistance in these bacterial isolates pose a growing threat to human health, necessitating continuous research into resistance mechanisms and methods to curb their spread [2].

In 2023, the World Health Organization (WHO) identified 12 types of antibiotic-resistant bacteria as a group of global research priority. Carbapenem-Resistant *Acinetobacter baumannii* (CRAB) was given special importance due to its ability to cause rapid outbreaks of infection [3]. This bacterium is part of the ESKAPE group of pathogens, which includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp* [4]. These antibiotic-resistant pathogens have a significant impact on public health, with millions of infections and thousands of deaths estimated annually [5]. Carbapenems belong to the β-lactam class of antibiotics, similar to penicillin's and cephalosporins, they work by inhibiting the formation of the bacterial cell wall, which leads to cell death. The widespread use of carbapenems has led to increased resistance to this class of antibiotics [6]. The study aims to investigate the latest developments in bacterial resistance to carbapenem for the year 2026, isolated from various infections.

2. Materials and Methods

A total of 140 clinical samples were collected from various sites of infections in patients of both genders and different age groups. The samples collected in Salah al-din Governorate, including Samarra General Hospital, Tikrit Teaching Hospital, and Al-Alam General Hospital, during the period from August 4, 2025, to December 10, 2025. 40 samples from urinary tract infections, 6 samples from sputum of patients with respiratory tract infections, 3 blood samples, 8 samples from wound infections, 25 samples from burns, 10 samples from stool, 10 samples from semen, 20 samples from vaginal, 10 samples from catheter, and 8

samples from body floods. The samples were cultured on differential and selective media (Himedia/India) for primary isolation, including blood agar, MacConkey agar and Mannitol Salt Agar, and incubated at 37°C for 24 hours. Additionally, diagnosis was performed using vitik 2 system.

Bacterial identification began with a visible examination of colonies growing on several kinds of media, such as blood agar and MacConkey's and mannitol salt agar. A battery of biochemical assays was then performed, including an oxidase assay yielding a blue/purple color development, a catalase assay where the addition of hydrogen peroxide formed gas bubbles in the test tube, a urease assay where the color of the medium changed from yellow to pink, an indole assay where the addition of Kovac's reagent formed a red ring around the edge of the medium, and methyl red and Voges-Proskaur assays that developed bright red color when indicators were added. The citrate assay showed a color change to blue, the triple sugar iron (TSI) assay detected gas production or a color reaction to oxygen depletion from growth of the tester organism. The motility test shows that microorganisms grow outward from the stab line in semi-solid agar. The coagulase test shows clustering of staphylococci in rabbit plasma within seconds after it's added to the medium. The hemolytic pattern is determined on blood agar which may exhibit alpha, beta or gamma lysis of red blood cells. The PYR test will produce a red color with *Streptococcus pyogenes*. The optochin sensitivity test is determined by zone of inhibition around a disk of optochin, while bile solubility is determined when the suspension becomes no longer cloudy. The novobiocin sensitivity test can differentiate among organisms by determining whether they are sensitive or resistant to novobiocin. Mannitol salt agar turns yellow if mannitol is fermented. The CAMP test results in a crescent shape (arrowhead) of increased hemolysis surrounding a line streaked with *S. aureus* which indicates the presence of *S. agalactiae*.

2.1 Diagnostic bacteria using VITEK 2 Compact

Start by preparing a bacterial suspension from an 18 to 24-hour pure culture in 0.45% saline, then adjust the turbidity to match a McFarland standard of 0.5 to 0.63 for identification or 0.5 to 0.55 for susceptibility testing and vortex well. Select the appropriate identification or susceptibility testing card based on the organism type, then place the card in the filling station where vacuum pulls the inoculum into all wells before sealing automatically. Load the card cassette into the VITEK 2 Compact instrument which incubates at 35.5 degrees Celsius and reads each well every 15 minutes using colorimetry and turbidimetry. The software analyzes the data to provide identification with probability percentage and determines minimum inhibitory concentrations interpreted as susceptible, intermediate, or resistant. Results are available in 2 to 10 hours for identification and 6 to 16 hours for susceptibility testing, then reviewed and reported to the laboratory system.

2.2 Screening of bacterial isolates for carbapenemase Production

2.2.1 Hodge test

A microbiological method used to detect the production of ESBL or carbapenems in bacteria by inoculating Mueller-Hinton agar with a sensitive indicator strain (e.g., *E. coli*), then a carbapenem antibiotic disk (like imipenem) is placed in the center. The test bacteria are heavily streaked from the edge of the plate towards the center disk, and after incubation, observing a cloverleaf-like or enhanced growth at the intersection of the line and the inhibition zone, which indicates a positive test.

2.3 DNA Extraction and PCR Amplification

DNA was extracted from bacterial isolates using the ABIOpure™ DNA extraction kit for Gram-negative bacteria. These isolates were selected because they showed resistance to carbapenem antibiotics during the susceptibility testing**.** The extraction was performed at central Laboratories of the Science College at Tikrit University**.**
The purity and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA) at 260 nm. Table 1 shows the primers used in the study, along with the molecular size for each gene and their sources.

Table 1- Primers Used in PCR Reaction

Gene	Primers	Amplicon Size (bp)	Reference
blaKPC	**F:** TCGCTAAACTCGAACAGG **R:** TTACTGCCCGTTGACGCCCAATCC	785	[7]
blaOXA-48	**F:** GTTTGGTCGCATATCGCAAC **R:** AATGCGCAGCACCAGGATA	436	[8]
blaVIM-type	**F:** ATTATCTACAGCAGCGCCAGTG **R:** TGCATCCACGTCTTTGGTG	296	[7]
blaOXA-1	**F:** CAC CTG GCA GAT CGG CAC **R:** CTG GCG CCC GGA TGC GG	527	[9]
blaL1	**F:** TGTTTTTGGTGGCATCGAT **R:** GTAAMRATGCTTGGTTCGC	179	[10]

PCR amplification was performed in a thermocycler (Bioneer, Korea) to detect carbapenem resistance genes. Each 25 µL reaction mixture contained 12.5 µL of master mix, 1 µL of each primer, 5 µL of DNA template, and 5.5 µL of nuclease-free water. The thermal cycling conditions was described in table 2. PCR products were analyzed by electrophoresis on a 0.8% agarose gel stained with ethidium bromide, and visualized under a UV transilluminator (Amersham, USA). Gel images were captured using a digital documentation system.

Table 2- PCR Program Steps

Step	Temperature (°C)	Time (min:sec)	Number of Cycles
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	According primer type	00:30	30
Extension	72	01:00	30
Final Extension	72	07:00	1
Hold	10	10:00	1

3. Results and Discussion

3.1 Bacterial Distribution of the Studied Isolates

According to the analysis performed on the results, *E.coli* was found to be the most common bacterium isolated, representing 27.18% of the isolate total, while *S. aureus* was the second most common bacterium represented isolating at 18.45%. The presence of this pathogen could likely cause a number of different infections. The overall incidence of *K. pneumoniae* was 6.80%. *K. pneumoniae* contributes significantly to respiratory and urinary tract infections; it is also responsible for many types of opportunistic infections that occur in hospitals and other healthcare facilities. Other bacterial species such as *A. baumannii* (3.8%),

Citrobacter spp., *P. aeruginosa, S. maltophilia* (3.8%), *E. cloacae* (2.9%) and Streptococcus pneumoniae (2.9%) had lower incidence rates than *K. pneumoniae*. The less prevalent species, such as *B. cepacia*, *P. vulgaris*, and *S. pyogenes* and *K. kristinae* at 1.9%, along with numerous other species appearing at low rates 0.97% constituted the remaining diversity.

Table 3- Bacterial Distribution of the Studied Isolates patterns. (n=103)

Bacterial Isolate	No.	%
Escherichia coli	28	27.1
Staphylococcus aureus	19	18.4
Klebsiella pneumoniae	7	6.8
Staphylococcus epidermidis	5	4.8
Streptococcus agalactiae	5	4.8
Acinetobacter baumanii	4	3.8
Citrobacter spp.	4	3.8
Stenotrophomonas maltophilia	4	3.8
Enterobacter cloacae	3	2.9
Pseudomonas aeruginosa	4	3.8
Streptococcus pneumoniae	3	2.9
Kocuria kristinae	2	1.9
Burkholderia cepacia	2	1.9
Proteus vulgaris	2	1.9
Streptococcus pyogenes	2	1.9
Klebsiella oxytoca	1	0.9
Pantoea spp.	1	0.9
Streptococcus mutans	1	0.9
Streptococcus viridans	1	0.9
Enterococcus casseliflavus	1	0.9
Staphylococcus intermedius	1	0.9
Staphylococcus auricularis	1	0.9
Staphylococcus capitis	1	0.9
Staphylococcus lentus	1	0.9

3.2 The phenotype detection

A number of different biochemical tests and observations of growth were completed to identify 11 different organisms which all belonged to the Gram negative aerobic bacteria group. The Gram negative organism *E. coli* demonstrates being gram negative (as rod-shaped, motile and oxidase negative), was found to be indole positive and methyl red positive, but was tested negative for Voges-Proskauer, citrate and urease. The fermentation of lactose and gas production would indicate an acid/acid with gas reaction for *E. coli* on TSI Agar. *K. pneumoniae* is a non-motile, encapsulated Gram-negative rod. It is negative for indole and methyl red but positive for Voges-Proskauer, citrate, and urease, and it ferments lactose with gas on TSI. *A. baumanni* is a non-motile Gram-negative coccobacillus that is negative for most tests except citrate, giving an alkaline/alkaline result on TSI. Citrobacter species is a motile Gram-negative rod with variable results for indole, urease, and lactose, but it is consistently positive for methyl red, citrate, and hydrogen sulfide production, showing acid/acid with H_2S on TSI. *Enterobacter cloacae* is a motile Gram-negative rod that is

positive for Voges-Proskauer and citrate but negative for indole and methyl red, and it ferments lactose with gas. *P. aeruginosa* is a motile Gram-negative rod that is oxidase and citrate positive, but negative for all other common tests, giving an alkaline/alkaline TSI result. *B. cepacia* is a non-motile, oxidase-positive rod that is positive for Voges-Proskauer and citrate, with an alkaline/alkaline TSI. *S. maltophilia* is a motile, oxidase-negative rod that is positive for citrate and urease, also giving an alkaline/alkaline TSI. Proteus vulgaris is a motile Gram-negative rod that is strongly positive for indole, methyl red, urease, and hydrogen sulfide, giving an alkaline slant/acid butt with H_2S on TSI. *K. oxytoca* is very similar to *K. pneumoniae* but differs by being indole positive, while still being positive for methyl red, Voges-Proskauer, citrate, and urease, and it ferments lactose with gas. Finally, Pantoea species is a motile Gram-negative rod that is positive for Voges-Proskauer and variable for citrate, negative for indole and methyl red, and ferments lactose with gas on TSI.
Regarding Gram-positive bacterial isolates, results for thirteen organisms, all Gram-positive cocci, were recorded. *S. aureus* is a cluster-forming coccus that is catalase and coagulase positive, produces beta hemolysis, ferments mannitol on MSA, and is sensitive to novobiocin but resistant to optochin and negative for PYR and CAMP. *S. epidermidis* does not produce any coagulase, does not produce any hemolysis and does not ferment mannitol, and is therefore susceptible to novobiocin, in contrast with *S. intermedius*, which has the same characteristics as *S. aureus,* except that *S. intermedius* ferments mannitol and is CAMP negative. *S. auricularis* is coagulase negative and shows gamma hemolysis, with variable susceptibility to novobiocin. *S. capitis* and *S. lentus* are both coagulase negative, and *S. lentus* is unique in its ability to grow in 6.5% bile salts and resist novobiocin. As far as streptococci are concerned, *S. pyogenes* is catalase negative and therefore PYR positive, has beta-hemolytic activity and is susceptible to optochin; on the other hand, *S. agalactiae* is also beta-hemolytic, but is PYR negative and CAMP positive. *S. pneumoniae* grows in pairs, is alpha-hemolytic, and is susceptible to optochin; *S. mutans* shows variable hemolytic activity and ferments mannitol. Viridans streptococci are generally alpha-hemolytic, are resistant to optochin, and do not grow in bile salts. *K. kristinae* is gamma hemolysis, coagulase negative, catalase positive, sensitive to novobiocin which does not ferment mannitol; *E. casseliflavus* is both 6.5% bile salts and PYR positive.

3.3 Antibiotic sensitivity test

Although *Escherichia coli* was the most frequently isolated pathogen, accounting for 27.18% of the total isolates in the studied microbial community, it showed the lowest resistance level among the five tested species, with only 17.8% of its isolates being resistant. This indicates that despite its high prevalence, the majority of *E. coli* strains in this sample remained susceptible to the antibiotic in question.

This pattern aligns with findings from a German study which reported that resistance rates of *E. coli* to carbapenems such as meropenem remained relatively low (below 10%) in community-acquired isolates in Europe, although higher in healthcare settings [11].
Susceptibility is preserved in *E. coli* to MEM and IPM both 17.8%. This aligns with [12], who reported resistance rates in the Middle East frequently exceed 40%. Opposing studies include [13] in New York, who found carbapenem resistance remained below 5% and MDR rate only 25% in community hospitals.
Susceptibility is preserved to MEM and IPM in *K. pneumoniae* (7 isolates). This confirms its reputation as a high-risk nosocomial pathogen. Supporting studies include [14], who highlighted the pandemic spread of carbapenem-resistant clones. *P. aeruginosa* (4 isolates) shows susceptibility to MEM (50%) and IPM (50%). A study conducted by [15] who found carbapenem resistance only 18% and MDR 31% in North America.
A. baumanii (4 isolates) exhibits 50% resistance to carbapenems. This offers a real-world example of the "nightmare bacterium [16] confirmed 95% of *A. baumannii* in the Middle East were carbapenem-resistant[17] reported carbapenem resistance in the US was 52%.

S. maltophilia (4 isolates): Shows 100% resistance to antibiotics. Sánchez (2015) confirmed intrinsic resistance to carbapenems, aminoglycosides, fluoroquinolones, and cephalosporins.

In stark contrast, alarmingly high levels of resistance were observed in the other species. *K. pneumoniae* showed an 85.7% resistance rate (6 out of 7 isolates), while *A. baumannii*, *P. aeruginosa,* and *S. maltophilia* each exhibited a 100% and 50% resistance rate respectively. This reflects the widespread prevalence of resistant strains, particularly among Gram-negative bacteria notorious for multidrug resistance, posing a serious therapeutic challenge. These results are consistent with warnings from the World Health Organization (WHO), which classifies some of these bacteria (such as carbapenem-resistant A. baumannii and P. aeruginosa) in its priority pathogen list for urgent research and development of new antibiotics due to critically limited treatment options [18].

Despite this, a new study from Saudi Arabia recently published in the Saudi Journal of Infectious Diseases in 2023 reported lower rates of resistance to K. pneumoniae and P. aeruginosa for urine samples from outpatients (24.6% and 15.2%, respectively) than those found herein (85.7% and 100%, respectively). This difference in the current data is likely due to different sources of the isolates used in the study. It is likely that the isolates in the current table originated from healthcare-associated environments (hospitals) or patients with complex infections, where the selective pressure from antibiotic use is higher, consequently leading to a greater prevalence of resistant strains. This hypothesis is supported by an Egyptian study conducted in intensive care units, which found resistance rates reaching 72.5% for *A.baumannii* and 68.4% for *P. aeruginosa* against a panel of antibiotics, underscoring the nature of healthcare-associated infections [20].

Table 4 -Antibiotic sensitivity test

Bacterial Isolate	No.	MEM No.%	IPM No.%
Escherichia coli	28	5/17.8	5/17.8
Staphylococcus aureus	19	0	0
Klebsiella pneumoniae	7	6/85.7	6/85.7
Staphylococcus epidermidis	5	0	0
Streptococcus agalactiae	5	0	0
Acinetobacter baumanii	4	4/100	4/100
Citrobacter spp.	4	0	0
Stenotrophomonas maltophilia	4	100	100
Enterobacter cloacae	3	0	0
Pseudomonas aeruginosa	4	2/50	2/50
Streptococcus pneumoniae	3	0	0
Kocuria kristinae	2	0	0
Burkholderia cepacia	2	0	0
Proteus vulgaris	2	0	0
Streptococcus pyogenes	2	0	0
Klebsiella oxytoca	1	0	0
Pantoea spp.	1	0	0
Streptococcus mutans	1	0	0
Streptococcus viridans	1	0	0
Enterococcus casseliflavus	1	0	0
Staphylococcus intermedius	1	0	0

Staphylococcus auricularis	1	0	0
Staphylococcus capitis	1	0	0
Bacterial Isolate	No.	0	0

3.2 Molecular Detection of Carbapenem-Resistance Genes in Diverse Bacterial Isolates

DNA was isolated from 19 isolates of *E. coli*, *K.pneumoniae*, *P. aeruginosa*, *A. baumannii*, and *S. maltophilia*., the DNA samples were subjected to agarose gel electrophoresis prior to their use in PCR to assess quality, which varied from 1.6 to 1.8.

3.3 Molecular detection

The following genes were amplified: *blaL1*, *blaKPC*, *blaOXA-48*, *blaVIM*-type, and *blaOXA-1*. The results varied regarding the presence of these carbapenem resistance genes, as observed in the table 4.

Table 4- Detection of Beta-Lactamase Resistance Genes in Clinical Bacterial Isolates using PCR

Bacterial Isolate	No	Target Gene	Positive Isolates	Percentage (%)
***Klebsiella* spp.**	6	blaKPC	4	66.7
A. baumanii	4	blaOXA-48	2	50
P. aeruginosa	4	blaVIM-type	0	0.0
E. coli	5	blaOXA-1	4	80.0
S.maltophilia	4	blaL1	0	0.0

3.3.1 blaKPC gene

The results in Figure 4 showed the presence of the *blaKPC* gene in 4 from 6 isolates of *Klebsiella* bacteria, at a rate of 66.7%, as shown in the table 4. The results consistence with those found in Iraq [21] where the prevalence of this gene was(62.5) %. However, this study differed from the study conducted in India [22] where the percentage of isolates carrying the gene was (20) %. As for samples 5 and 6, they did not appear in the results, indicating that these isolates do not carry the gene (blaKPC).

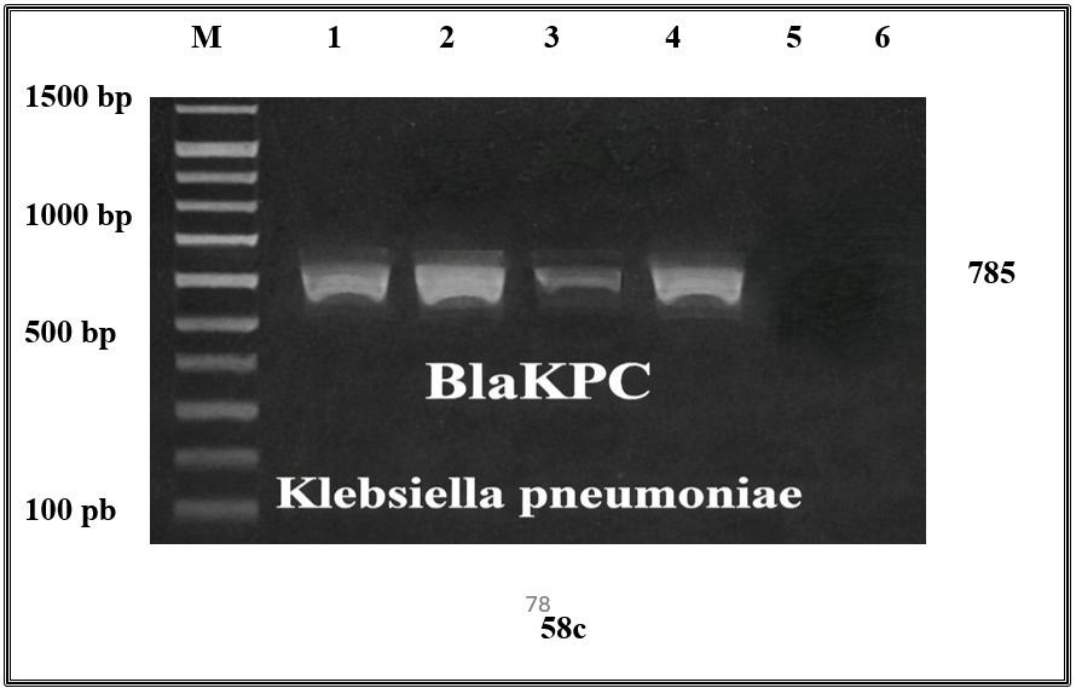

Figure -1 Agarose gel electrophoresis image showing PCR amplification products of the *blaKPC* gene in *Klebsiella penemonia* isolates, a distinct band appears at 785 bp in all tested samples Except 5 and 6 they did not appear in the result indicating that these isolates do no carry the gene.

3.3.2 blaOXA-48 gene

The results in Figure 1 showed the presence of the *blaOXA-48* gene in 2 isolates from 4 of *A. baumanii*, at a rate of 50.0%%.

The results are less than that found by [23] where the prevalence of this gene was(6.7) %. However, this study differed from the study conducted in Iran [24] where the percentage of isolates carrying the gene was (2.3) %. As for samples 1 and 2, they did not appear in the results, indicating that these isolates do not carry the gene *blaOXA-48*. The emergence of these high rates of carbapenem resistance is attributed to several reasons. The first is the small number of samples that were diagnosed. Additionally, the samples were collected from the intensive care unit where there was an outbreak of the bacteria. This is a serious indicator that must be heeded, despite ongoing sterilization and disinfection procedures. These bacteria now show virulence in resisting most disinfectants used in sterilization.

This concern is supported by scientific research. A study by[25] found that *A. baumannii* strains isolated from ICUs during an outbreak showed not only high-level carbapenem resistance but also a significantly increased tolerance to common hospital disinfectants. This dual resistance highlights the bacteria's adaptability and the challenges in controlling its spread, even with rigorous infection control protocols. The authors suggest that the overuse or misuse of certain disinfectants may contribute to the selection of strains with enhanced biocide tolerance, which is often linked to multidrug resistance mechanisms.

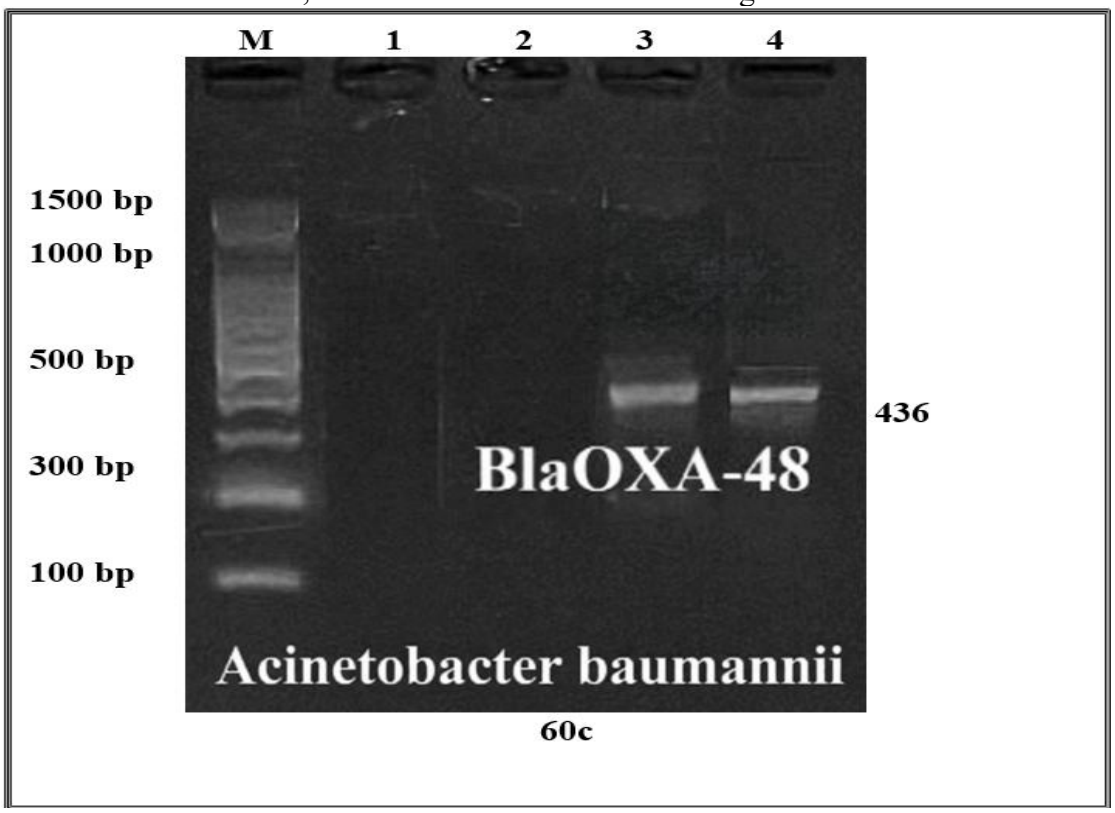

Figure -2 Agarose gel electrophoresis image showing PCR amplification products of the *blaOXA-48* gene in *Acinetobacter baumannii* isolates, a distinct band appears at 436 bp in 3 and 4 tested samples but 1 and 2 they did not appear in the result indicating that these isolates do not carry the gene.

3.3.3 blaVIM-type gene

The products of the *blaVIM*-type gene in all *P. aeruginosa* isolates doesn't show bands indicating that these isolates do not carry the gene. However, this study differed from the study conducted in Iran [26] where the percentage of isolates carrying the gene was (40-30) %.

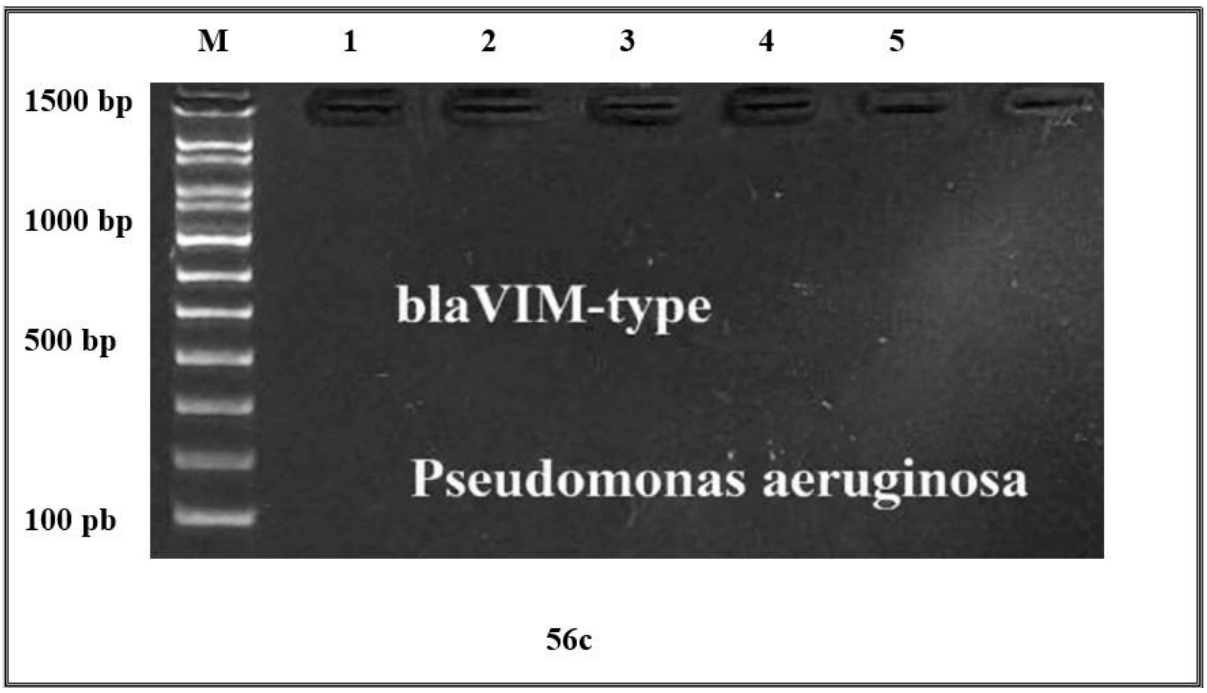

Figure -3 Agarose gel electrophoresis image showing PCR amplification products of the *blaVIM*-type gene in *Pseudomonas aeruginosa* isolates, all samples dosen't show bands indicating that these isolates do not carry the gene.

3.3.4 blaOXA-1 gene

The results in Figure 1 showed the presence of the *blaOXA-1* gene in 4 from 5 isolates of *Escherichia coli* bacteria, at a rate of 80 %. The result is similar to those found by [27] where the prevalence of this gene was(89.7) %. However, this study differed from the study conducted in Iran [28]. where the percentage of isolates carrying the gene was (10-30) %.

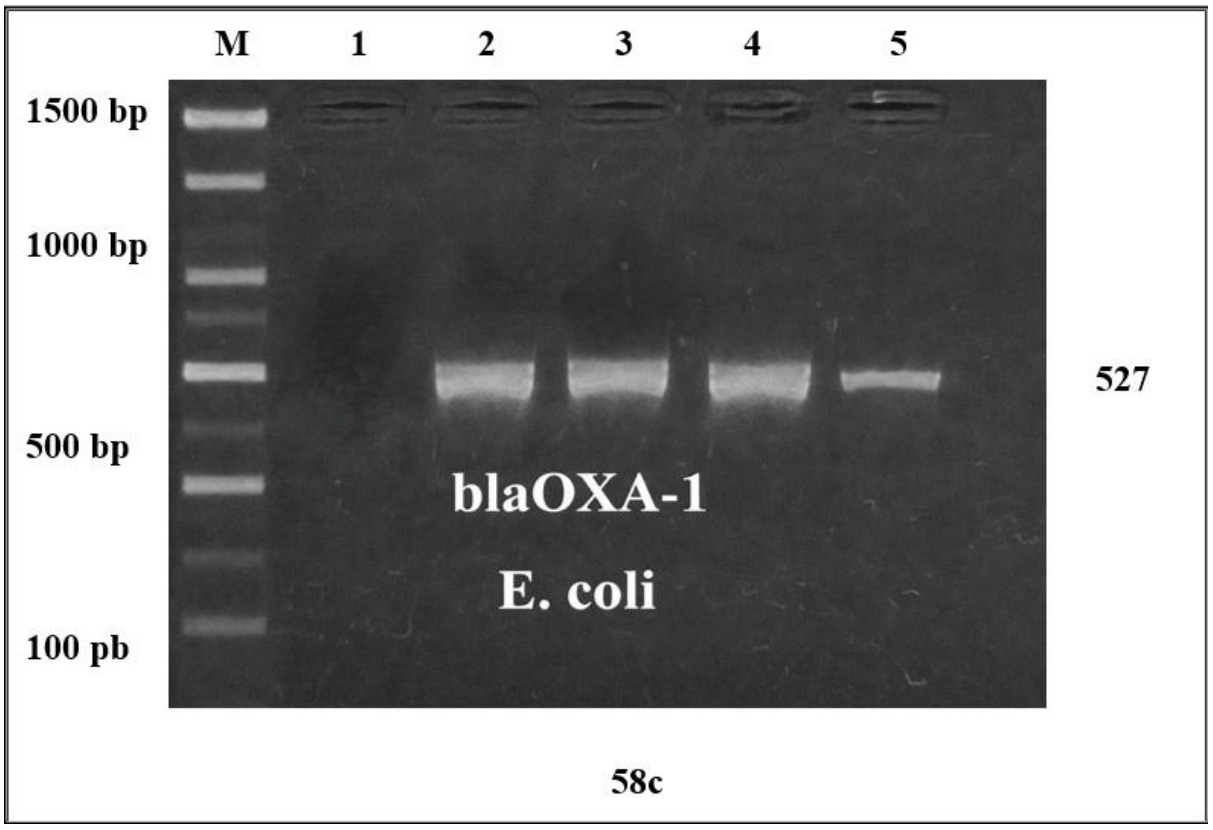

Figure -4 Agarose gel electrophoresis image showing PCR amplification products of the *blaOXA-1*gene in *Escherichia coli* isolates, a distinct band appears at 527 bp in all tested samples, except 1 it did not appear in the result indicating that this isolate does not carry the gene.

3.3.5 blaL1 gene

The results in Figure 1 showed no presence of the *blaL1* gene in 4 isolates of *S. maltophilia.* However, this study differed from the study conducted in Iraq [29] where the percentage of isolates carrying the gene was (100) %.

The failure to detect the gene responsible for carbapenem resistance in some isolates by PCR, despite the isolates demonstrating resistance during antimicrobial susceptibility testing, can be explained scientifically by the fact that the absence of gene bands does not necessarily indicate the absence of resistance may possess intrinsic resistance mechanisms, including the production of L1 and L2 enzymes, the activity of efflux pumps, and reduced membrane permeability [30]. Such mechanisms may remain undetected when the PCR assay is designed only to identify common acquired carbapenemase genes. Furthermore, the lack of amplification bands may also result from technical limitations, such as poor DNA quality, the presence of PCR inhibitors, unsuitable primer design, or suboptimal reaction conditions [31].

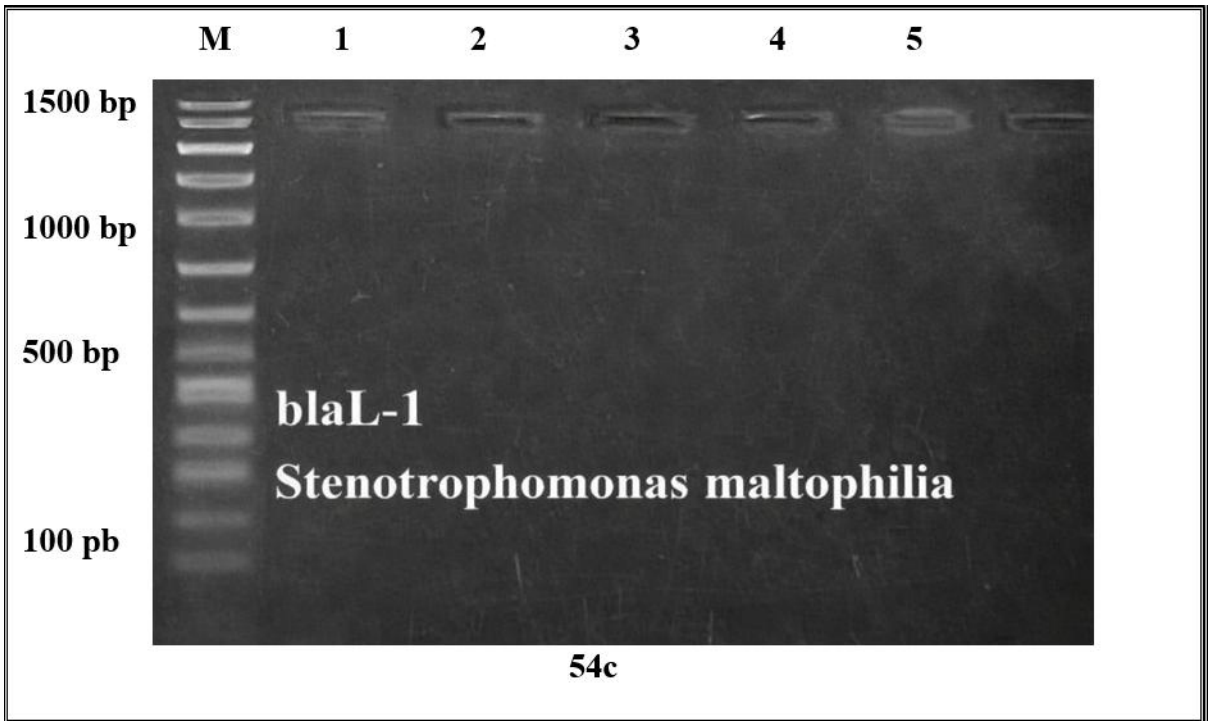

Figure -5 Agarose gel electrophoresis image showing PCR amplification products of the *blaL1*gene in *Stenotrophomonas maltophilia* isolates, all samples dosen't show bands indicating that these isolates do not carry the gene.

4. Conclusion

This study demonstrated that although most bacterial isolates remained susceptible to carbapenem antibiotics, a significant proportion of specific isolates exhibited resistance to these agents. Phenotypic detection methods confirmed carbapenem resistance among selected isolates, while molecular analysis revealed the presence of carbapenem-resistance genes associated with this resistance pattern. The coexistence of phenotypic resistance and resistance-associated genes highlights the potential dissemination of carbapenem-resistant bacteria in clinical settings. These findings emphasize the importance of continuous antimicrobial susceptibility testing, early molecular detection of resistance genes, and the implementation of effective infection control strategies to limit the spread of carbapenem-resistant pathogens and preserve the efficacy of carbapenem antibiotics.

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