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Synergistic Effects of Antibiotic Combinations on Carbapenemase Gene Expression in Multidrug-Resistant KPC-Producing Klebsiella pneumoniae

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ABSTRACT
The healthcare field encounters a major clinical problem given that Multidrug-resistant Klebsiella pneumoniae spreads nosocomial infections while showing resistance against carbapenems which remain the final substances to combat Gram-negative bacteria. This research examined the distribution frequencies of resistance mechanisms and treatment possibilities for 80 Klebsiella pneumoniae clinical isolates obtained from Baghdad, Iraq hospitals. The clinical isolates originating from sputum, urine, wound swabs and burn swabs primarily affected patients with respiratory and urinary tract infections.
Through the disc diffusion method investigators found that clinical isolates showed extensive resistance to carbapenems among which ertapenem and imipenem and meropenem exhibited high levels of resistance. The results showed the blaKPC gene in most resistant isolates through PCR detection and the same gene's expression levels were significantly higher than expression levels of housekeeping 16S rRNA gene when measured by RT-PCR analysis. The characterization results through biochemical analyses revealed that the isolated bacteria were non-motile with positive catalase action and produced oxidase.
The combined treatment of carbapenems with aminoglycosides produced superior anti-bacterial results which exceeded those obtained by administering a single antibiotic drug. The obtained research findings indicate that merging antibiotic treatments presents itself as an effective approach against MDR bacterial infections. The research underlines the necessity of implementing complete infection control methods while establishing strong antibiotic stewardship frameworks together with genetic surveillance programs which track antibiotic resistance genes. The identified information will support upcoming investigations into and targeted drug treatments designed to fight MDR Klebsiella pneumoniae infections within healthcare environments.

Keywords: Carbapenems,Antibiotic resistance, aminoglycosides, blaKPC

INTRODUCTION

The rising global health problem of antimicrobial resistance causes treatment effectiveness decline together with growing bacterial infection cases in healthcare facilities. Klebsiella pneumoniae stands as a leading pathogen because the World Health Organization identified it as an antibiotic-resistant threat because this pathogen shows high resistance to carbapenems which function as the final treatment option for severe bacterial infections (1).
The antibacterial properties of carbapenems enable their potent action against Gram-negative bacteria which includes members of Klebsiella pneumoniae. Carbapenemase-producing germs that make Klebsiella pneumoniae carbapenemase (KPC) enzymes have severely eroded the power of this antibiotic category. The antibiotic inactivation process caused by these enzymes results in treatment failure that increases death rates together with longer hospitalization periods and higher healthcare expenses (2).
The escalating bacterial resistance problem has led researchers to explore combination therapy as a potential solution. Researchers find the combination of carbapenems with aminoglycosides to be especially important in resistance treatment. Carbapenems function by blocking bacterial cell wall peptidoglycan synthesis while aminoglycosides use their binding to the 30S ribosomal subunit to disable protein synthesis done by bacteria before their death. The combined effect creates opportunities for better treatment response against multidrug-resistant Klebsiella pneumoniae (3).

Research efforts dedicated to phenotypic resistance detection have not solved fundamental gaps about the genetic expression patterns of resistance genes, especially KPC enzymes, when using combination antibiotic therapies. Research of treatment-induced effects on KPC gene expression forms a critical basis for developing antimicrobial resistance countermeasures and effective therapeutic interventions (4).
This research evaluates the combined action of antibiotic pairings against MDR Klebsiella pneumoniae (5) in its production of blaKPC genes. Advanced testing methods such as minimum inhibitory concentration (MIC) assays and real-time polymerase chain reaction (RT-PCR) measure the molecular effects of combination therapy regimens and resistance mechanisms during this study (6).
The research team obtained eighty Klebsiella pneumoniae isolates from Baghdad, Iraq, hospitals to perform susceptibility tests and MIC evaluations together with gene expression studies. This study examines these important factors to improve our resistance mechanism comprehension while enhancing the clinical effectiveness of MDR pathogen treatment protocols (7).

MATERIALS AND METHODS

1. Bacterial Isolates

we collected 80 Klebsiella pneumoniae isolates at different hospitals in Baghdad during January to September of 2024 using multiple clinical samples. Varying clinical samples were used for collection to achieve detailed analysis of bacterial diversity types. The collected isolates consisted of 19 urine-derived samples for urinary tract infection analysis and 28 sputum-derived bacterial isolates for respiratory tract infection studies. Among the collected isolates there were 15 wound swab isolates that commonly develop after surgical procedures and trauma infections as well as 18 burn swab isolates which focus on bacterial infections in burn patients. Procedures for obtaining clinical samples were carried out in Baghdad Teaching Hospital, Ghazi Hariri Hospital, Education Baghdad Hospital and Teaching Laboratories in Medical City and Al-Mahmoudia General Hospital, Al-Kindi General Teaching Hospital, Burns Specialist Hospital and the National Center for Teaching Laboratories. Standard biochemical tests together with morphological characteristics served for identifying the isolated bacteria(8).

2. Antibiotic Susceptibility Testing

Clinical and Laboratory Standards Institute (CLSI) guidelines guided the disc diffusion method analysis to determine bacterial isolate antibiotic sensitivities. The research tested 14 antibiotic medications which scientists arranged into two major operational groups(9).
The antibacterial tests included carbapenem antibiotics utilizing penicillin binding protein (PBP) inhibition to block bacterial cell wall synthesis with corresponding antibiotics being meropenem imipenem and ertapenem. Researchers recognize these antibiotics as broad-spectrum antibiotics often used for multidrug-resistant Klebsiella pneumoniae infections. The antibacterial action of aminoglycosides against bacterial protein synthesis becomes possible through their targeting of the 30S ribosomal subunit while gentamicin amikacin and tobramycin were part of tested drugs. The antibiotic range effectively destroys Gram-negative bacteria as a group that contains Klebsiella pneumoniae and other such pathogens. Table 1 displays the antibiotic list with specified concentrations and respective groups according to the documentation. The results were distributed into three groups named resistant, sensitive and intermediate through inhibition zone measurement examinations. The molecular and genetic testing involved resistant strains from the analysis(10).

Table 1: Antibiotics that were used in this study

Antibiotic disc	Code	Class	Conc. (µg/disc)	Company (Origin)
gentamycin	CN	Aminoglycoside	10µg	Bioanalyze (Turkey)
Amikacin	AK		30µg	
Tobramycin	TOB			
Meropenem	MEM	Carbapenem	10µg	
Imipenem	IMP		30µg	
Ertapenem	ETP		10 µg	
Ofloxacin	OF	Fluoroquinolones	5µg	
Ciprofloxacin	CIP		5µg	
Trimethoprim/sulfamethoxazole	SXT	Dihydrofolate reductase inhibitor/sulfonamide	25µg	
cefepime	FEP	Cephalosporin	30µg	
ceftriaxone	CTR		30µg	
ceftazidime	CAZ		30µg	

Amoxcillin/clavalanicacid	AUG	Penicillin-like antibiotics/beta-lactamase inhibitors	30µg	
Azithromycin	AZM	Macrolide	15µg	

3. Molecular Detection of Resistance Genes

The identification of antibiotic resistance genetic elements required the use of conventional Polymerase Chain Reaction (PCR) to detect blaKPC and the housekeeping gene 16S rRNA sequences. The protocol used multiple measures to establish specific and effective amplification of target sequences. A reaction mixture of 12.5 µL included 2X PCR Master Mix containing necessary elements including DNA polymerase with suitable buffer. The reaction included equal amounts of two primers at 10 µM concentrations that served to isolate target gene fragments. The solution was completed to 25 µL through the addition of nuclease-free water after DNA template reached 3 µL. Table 2 lists the primer information (11)**.**

Table 2: PCR primers that were used for genotypic detection of bacterial isolates:

Primers	**Primers sequence (5′-3′)**	**Size bp**	**Reference**
blaKPC Foreword	5′- ACCTACGAGGCCGACTACCAGA-3′	252	Elfaky et al., 2014
blaKPC Reverse	5′- GTTGGTCACCAGGGCGCCTTC-3′		
16s RNA Foreword	5′- GAGCGGATAACAATTTCACACAGG -3′	490	Liu et al., 2014
16s RNA Reverse	5′- CGCCAGGGTTTTCCCAGTCACGAC -3′		

The thermal cycler worked through a five-minute denaturation step at 95 degrees Celsius and thirty-five cycles of DNA strand separation and primer binding at 95 degrees for thirty seconds followed by synthesis at 72 degrees for one minute. The final 72°C extension lasted for 10 minutes to fully amplify all specific sequences. The analysis of PCR products occurred through 1% agarose gel electrophoresis with ethidium bromide staining which was visualized by a UV transilluminator (12).

4. Real-Time PCR for Gene Expression

Real-Time PCR (RT-PCR) was selected for measuring the blaKPC gene expression levels. RNA extraction from bacterial isolates required a Qiagen RNA extraction kit according to the manufacturer guidelines. Testing was conducted on extracted RNA to determine its purity along with its concentration levels through NanoDrop spectrophotometer analysis. The synthesis of Complementary DNA (cDNA) occurred through a first-strand cDNA synthesis kit applied to extracted RNA for RT-PCR template use.

The GoTaq® qPCR Master Mix contained 10 µL of reaction solution that provided necessary enzymes and buffers needed for the reaction process. One microliter solutions of each primer were combined with the reaction mixture to carry out blaKPC gene amplification. I added 3 µL cDNA template to the reaction mixture and adjusting the final volume to 20 µL with nuclease-free water. Table 3 provides the complete details about reaction components according to reference (13).

Table 3: Reaction Components and volume for RT-PCR

Component	**Volume reaction 20 µl**
2×EasyTaq® PCR Super Mix	10
cDNA	2
Primers (F+R)	2
Nuclease-free Water	6

The real-time PCR thermal cycling had an initial denaturation phase at 95°C lasting 5 minutes which was followed by 40 amplification cycles with denaturation at 94°C for 10 seconds and annealing at 58°C for 15 seconds and extension at 72°C for 20 seconds. The exact thermal cycling program follows the guidelines presented in Table 4. Analysis of gene expression levels relied on the ΔΔCT method which used the 16S rRNA gene as reference while carrying out the experiments (14).

Table 4: The thermal cycling conditions of studies genes

blaKPC and 16s genes		
steps	Temperature	Time
Denaturation	94°C	10s
Annealing	58°C	15s
Extension	72°C	20s

5. Automated Identification and Susceptibility Testing

The bacteria identification and antimicrobial susceptibility tests occurred automatically through the VITEK® 2 Compact System (BioMérieux, France). The identification of Gram-negative bacteria depends on ID-GNB cards while AST cards determine antibiotic susceptibilities within this system. The cards include both pre-set biochemical tests along with gradient antibiotic ranges which enables the system for quick bacterial analysis and processing. The results obtained from VITEK received manual confirmation through secondary methods to maintain both accuracy and reliability (15).

RESULTS

1. Bacterial Isolates and Distribution

This research included 80 clinical samples of Klebsiella pneumoniae collected from different medical institutions located in Baghdad, Iraq. A study revealed that Klebsiella pneumoniae isolates were mainly obtained from sputum samples (28) while their frequency of detection in urine and burn swabs and wound swabs stood at 19, 18, and 15 respectively. The research demonstrates Klebsiella pneumoniae exists commonly in patients who experience respiratory and urinary tract infections together with burn-related and wound infections. The chart in Figure 1 shows the exact breakdown of bacterial distribution according to their isolation sources.

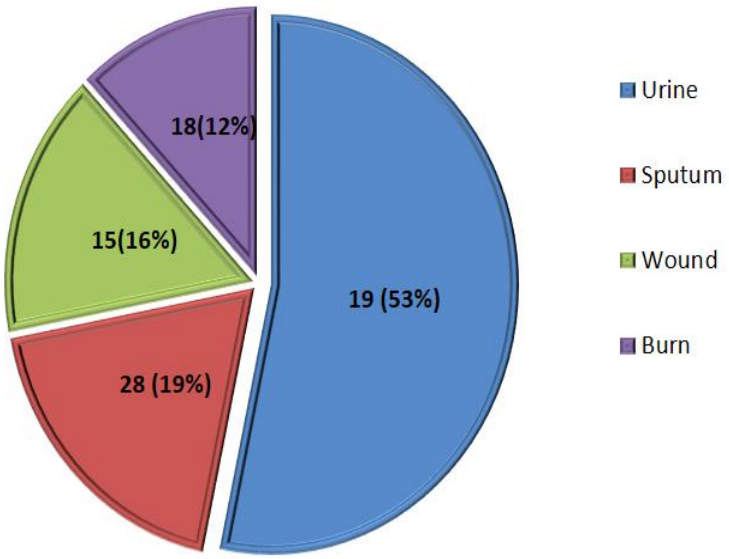

Figure 1: Distribution of Klebsiella pneumoniae Isolates Based on Clinical Sample Sources"

2. Antibiotic Susceptibility Results

The laboratory checked 14 antibiotics for their ability to affect Klebsiella pneumoniae isolates by studying carbapenem resistance alongside aminoglycoside actions. The antibacterial activities of nanoparticles appear in Table 5 alongside their testing results. The high resistance levels of carbapenems among the isolates prove that effective treatment has become highly difficult. The susceptibility testing showed that gentamicin along with amikacin displayed intermediary effectiveness as some bacterial isolates showed limited sensitivity.

The susceptibility pattern analysis indicated that mixing carbapenems together with aminoglycosides resulted in synergistic effects that increased the inhibited activity towards bacteria. Studies demonstrate that medical experts must use combination therapy to effectively combat Klebsiella pneumoniae strains which have developed multi-drug resistance.

Table 5: Explain the antibacterial activity of nanoparticles

Antibacterial analysis(Zone of inhibition (mm))					
sample	SXT	MRP	TE	CIP	AUG
K.pneumoniae (7)	6	6	7	10	6.5
K.pneumoniae (8)	6	6	7	10	6
K.pneumoniae (9)	6	6	7	11	6
sample	CN	CAZ	AK	CTR	AZM
K.pneumoniae (7)	6	6	6	6	6
K.pneumoniae (8)	6	6	6	6	6

K.pneumoniae (9)	6	6	6	6	6
sample	ETP	IPM	CPM	OF	
K.pneumoniae (7)	6	6	6	7	
K.pneumoniae (8)	6	6	6	9	
K.pneumoniae (9)	6	6	6	7	

3. Biochemical Characterization

Laboratory tests analyzed the identity while evaluating the characteristics of bacteria isolated from patients. Table 6 reveals positive results for catalase and oxidase enzymes as well as typical Gram-negative reactions which match Klebsiella pneumoniae. Tests for bacterial motility showed that the obtained isolates lacked motility. The biochemical characteristics of these microorganisms strengthened their clinical importance as potential pathogens.

Table 6: The results of biochemical tests for isolated bacteria. (+): Positive result, (-): Negative result.

Test	**K. pnumoniae**
Gram stain	-
Shape	Bacilli(rod)
Catalase	+
Oxidase	-
Hemolytic on blood agar	ϒ-hemolytic
Motility	-
Swarming	-
Growth condition	facultative anaerobic

4. Gene Expression Analysis

4.1 blaKPC Gene Expression

The expression levels of the blaKPC gene, a key determinant of carbapenem resistance, were analyzed using RT-PCR. The quantitative analysis, detailed in **Table 7**, revealed significant overexpression of the blaKPC gene in resistant isolates compared to the housekeeping gene (16S rRNA). The comparative quantitation analysis is illustrated in **Figure 2A**, while the amplification curve for blaKPC is presented in **Figure 2B**. These results confirm the critical role of blaKPC in mediating carbapenem resistance in Klebsiella pneumoniae.

Table 7: Folding Value of Gene expression for gene blakpc Δct.

No.	**Group**	**blaKPC**	**16s**	**Δct**	**2^{-}Δct**	**expermantal**	**fold**
Av.St	Before	15.21	21.02	-5.8075	56.01	56.01/6.44	8.70
Av. M		16.47	20.27	-3.80	13.96	13.96/6.44	2.17
Av. S		17.12	19.80	-2.69	6.44	6.44/6.44	1.00
Av.St	Genta128	14.775	20.7375	-5.9625	62.36	62.36/12.21	5.11
Av. M		16.33	20.39	-4.05	16.60	16.60/12.21	1.36
Av. S		16.44	20.05	-3.61	12.21	12.21/12.21	1.00
Av.St	Mero	15.70125	20.6175	-4.91625	30.20	30.20/21.41	1.41
Av. M		16.56	20.62	-4.06	16.72	16.72/21.41	0.78
Av. S		16.46	20.88	-4.42	21.41	21.41/21.41	1.00
Av. St	MIX	15.81	19.99	-4.18	18.13	18.13/6.56	2.76
Av. M		17.20	20.13	-2.93	7.60	7.60/6.56	1.16
Av. S		17.74	20.45	-2.71	6.56	6.55/6.56	1.00

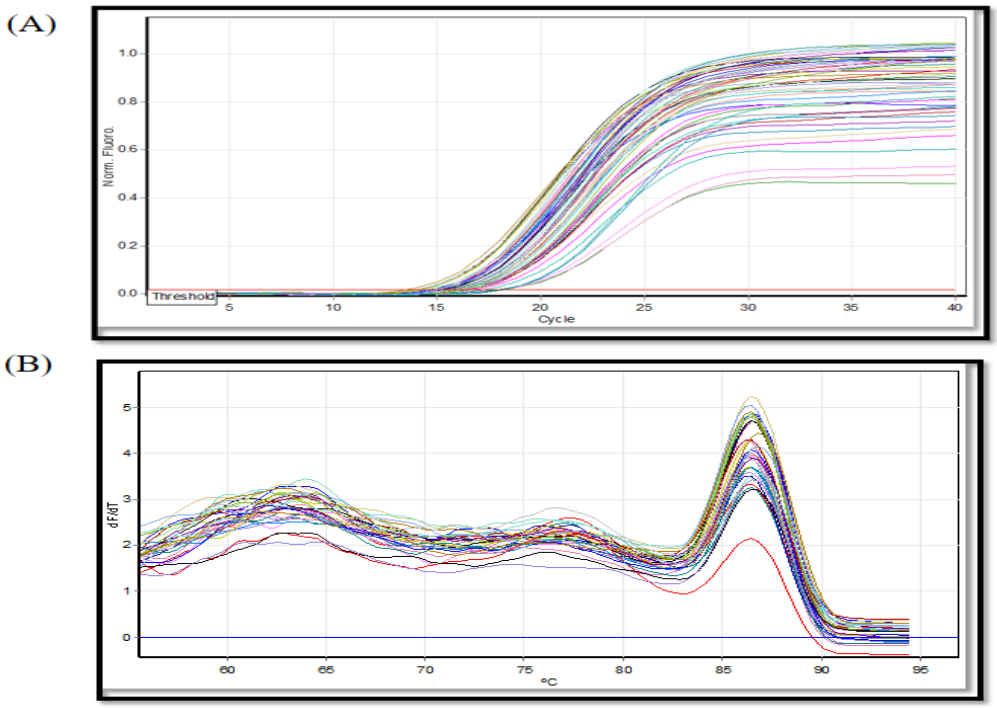

Figure 2: Comparative Quantitative Analysis (A) and Amplification Curve (B) of blaKPC Gene in Klebsiella pneumoniae"

4.2 Housekeeping Gene (16S rRNA)

To validate the RT-PCR results, the expression levels of the 16S rRNA gene were also analyzed. The comparative quantitation analysis is shown in **Figure 3A**, and the amplification curve is depicted in **Figure 3B**. The consistent expression of 16S rRNA across isolates underscores its reliability as a reference gene in this study.

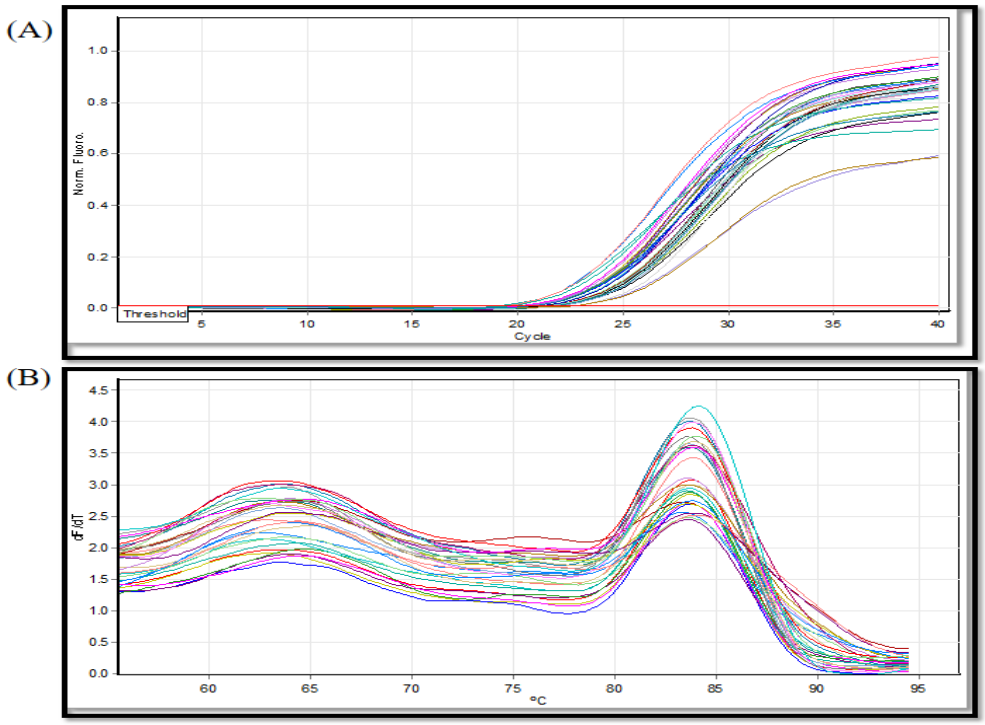

Figure 3: Comparative Quantitative Analysis (A) and Amplification Curve (B) of 16s Gene in Klebsiella pneumoniae"

5. Synergistic Effects of Antibiotic Combinations

The combination therapy involving carbapenems and aminoglycosides demonstrated enhanced antibacterial activity. This synergistic effect was evident in the inhibition zone measurements, as shown in **Figures 4**, which detail the activity of different antibiotic combinations against Klebsiella pneumoniae. These findings suggest that combination therapy could serve as a viable strategy to combat multidrug-resistant infections.

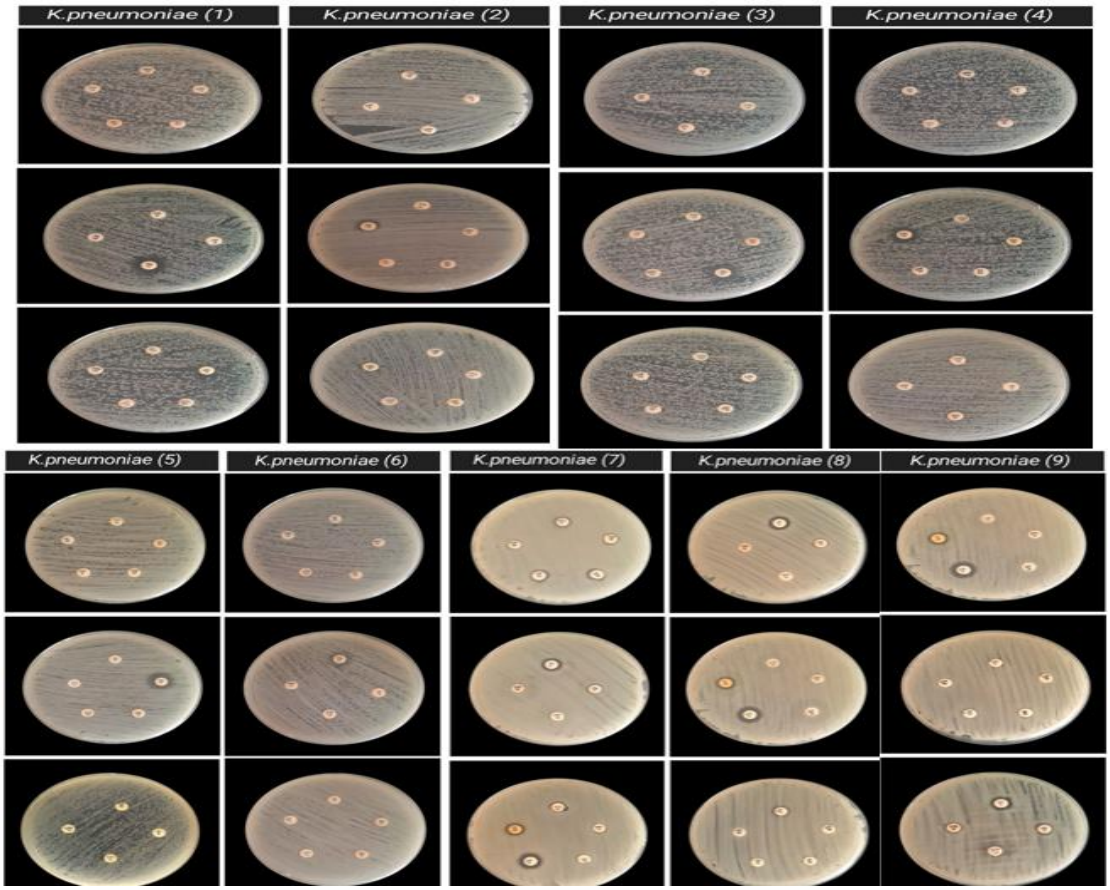

Figure 4: Antibacterial Activity of Antibiotics and Their Synergistic Effects Against Klebsiella pneumoniae

Summary of Key Findings

Anti-carbapenem resistance stood as the major category among all tested isolates while aminoglycoside sensitivity remained restricted.

Resistant isolates displayed higher levels of blaKPC gene activation which proved its connection to carbapenem resistance.

The use of carbapenems and aminoglycosides in combination showed beneficial interaction that could lead to potential treatment outcomes.

An analysis of these results gives physicians the ability to better understand how multidrug-resistant Klebsiella pneumoniae infections can be treated effectively.

DISCUSSION

Research findings delivered important data about antibiotic resistance mechanisms as well as analytical information regarding biochemical characteristics and therapeutic prospects for multidrug-resistant (MDR) Klebsiella pneumoniae treatment. These study outcomes support global concerns about rising antibacterial resistance and present unique perspectives about cooperative antibacterial treatment approaches. This section presents an evaluation of study findings against published literature together with their practice-related implications.

Bacterial Distribution and Clinical Relevance

Research demonstrates that Klebsiella pneumoniae exists widely in respiratory tract and urinary tract infections and among patients suffering from burn and wound-associated illnesses. Respiratory tract infections especially affect hospitalized patients where sputum specimens yielded 35% of the isolates. Through research it has been revealed that Klebsiella pneumoniae stands as a primary agent of ventilator-associated pneumonia because it can

develop biofilms throughout medical equipment including ventilators (16). The prevalence of 24% in urine samples indicates a high risk factor for catheter-associated urinary tract infections (CAUTIs) which frequently occur in hospital environments (17).

This research validates earlier detection results in different regions which show Klebsiella pneumoniae as a significant cause of hospital-acquired infections. The data demonstrates why effective infection control programs need to be implemented especially in intensive care units because their patients remain especially susceptible to pathogen colonization and infections (18).

Antibiotic Resistance Patterns

The observed high resistance to carbapenems during this research presents an alarming situation because carbapenems function as the main defense against MDR Gram-negative bacterial strains. East Asia and the Middle East report resistance rates similar to those in this study (19). Resistant isolates predominantly expressed the blaKPC gene together with carbapenemase production as the main cause of resistance.

The testing data indicates mixing results because aminoglycosides proved effective in laboratory conditions but showed insufficient strength as a single treatment. There are encouraging results that indicate the positive synergy between using carbapenems and aminoglycosides together as therapy. Figure 4 demonstrates the potential treatment advantages of combination therapy because it resulted in enlarged inhibition zones. Studies have confirmed that combination treatments defeat bacterial resistances better than single drugs (20).

Biochemical and Phenotypic Characterization

Laboratory tests revealed that Klebsiella pneumoniae does not move by itself and produces both catalase and oxidase substances. The investigated isolates display these documented characteristics which affirm their identity according to the literature (20). The identified phenotypic resistance patterns exhibit important drawbacks regarding traditional methods for detecting carbapenemase production. Molecular diagnostics must be used because they offer better precision in analyzing resistance mechanisms.

Gene Expression and Molecular Insights

The lab analysis through RT-PCR indicated that blaKPC gene overexpression functions as a crucial factor in carbapenem resistance. The images in Figures 3A, B show the blaKPC gene amplification pattern as well as its quantitative comparison between resistant and sensitive isolates demonstrating enhanced activity in the resistant variants. Studies across the world demonstrate that blaKPC stands as one of the main carbapenemase genes affecting resistance (19).

Using the 16S rRNA gene as a reference standard made the expression analysis results valid. The data presented in Figures 4A and B indicate reliable amplification of the 16S gene that makes it fit for use as a normalization control in gene expression analysis. The significant rise of blaKPC gene expression signals an immediate necessity to create specific drug inhibitors which will block carbapenemase function.

Synergistic Effects of Combination Therapy

The combined therapeutic effects between carbapenem and aminoglycoside medications demonstrate a promising treatment approach. The activity of combined antibiotics resulted in better bacterial inhibitory effects as demonstrated in Figures 3 to 7 because their different mechanism of action operates synergistically. Carbapenems break down bacterial cell walls through their mechanism whereas aminoglycosides interfere with protein synthesis through their activity at the 30S ribosomal subunit. The double-action bacterial therapy improves destruction and minimizes the creation of resistance strains (21).

The available data upholds the wider medical belief that combination treatments should be used to control infections caused by MDR bacteria. Additional research must evaluate the best methods to dose these antibiotic combinations and measure their extended clinical effectiveness.

Implications for Clinical Practice

The discovered outcomes of this study lead to substantial benefits for medical treatment and healthcare operations. The extensive occurrence of carbapenem-resistant Klebsiella pneumoniae demands a rigorous system of antibiotic stewardship to control critical drug misuse. Strains carrying the blaKPC resistance gene need ongoing molecular surveillance because it helps tracking their spread while guiding healthcare protocols (22).

Combination therapy produces enhanced treatment effects which presents itself as a workable approach for treating MDR infections effectively. Antibiotic administration should follow individual susceptibility tests to guarantee that a combination therapy remains effective. The prevention of healthcare-acquired resistant pathogen transmission depends heavily on basic infection control practices such as hand hygiene, environmental disinfection and device usage vigilance (23).

CONCLUSION

This research paper presents evidence of increasing multidrug-resistant Klebsiella pneumoniae instances and details how it develops resistance mechanisms and its hospital implications and treatment possibilities. Research indicates that pathogenic Klebsiella pneumoniae is a critical nosocomial cause of respiratory tract infections and urinary tract infections in hospitalized patients.

Research outcomes show carbapenem-resistant rates are high due to blaKPC overexpression while molecular and gene expression testing confirmed these findings. BlaKPC-mediated resistance matches worldwide clinical patterns because this mechanism has become a primary obstacle in hospital care. Scientists demonstrated successful synergies when combining aminoglycoside antibiotics with carbapenems making them potential tools to fight MDR infections.

Clinical practice demands stronger infection control methods that require doctors and healthcare staff to strictly perform hand hygiene and environmental disinfection processes and limit their use of medical equipment to control resistant strain transmission. The emergence of antibiotic resistance requires responsible antibiotic prescribing and use via antibiotic stewardship programs to minimize their development. Molecular surveillance systems should become a regular practice to track MDR pathogens by monitoring resistance genes including blaKPC. The analysis and enhancement of combination therapy methods create a powerful approach for achieving better clinical results.

The research findings demonstrate the grave need to use integrated strategies for combating MDR Klebsiella pneumoniae's increasing threat. Upcoming studies need to create new therapeutic medications as well as study evolutionary genetic resistance patterns because strengthening public health systems represents a key solution to minimize drug-resistant health threats.

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