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Synergistic effect of some antibiotics against multidrug resistant clinical isolates *Acinetobacter baumannii*

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Synergistic antibiotic combinations might offer an approach to overcome microbial resistance mechanisms. This work investigated genetic determinants of resistance to antibiotics. and evaluated *in vitro* the impacts of several different antibiotic combinations on a panel of carbapenem-resistant *Acinetobacter baumannii* (CRAB) and the extended drug-resistant *A. baumannii* (XDR – *A. baumannii*) strains. Clinical samples were obtained from patients hospitalized in the Intensive Care Unit (ICU) from August 1, 2024, to August 31, 2025. The "BD Phoenix Automated System" was employed to identify isolated bacteria at species-level, and for determination of the blaOXA-51-like gene. The isolates were screened for some carbapenemase genes and the ISAba1gene using PCR. The fractional inhibitory concentration index (FICI) was used to detect the effect of the various antibiotics used in combination. The study used 24 strains of *A. baumannii*. All tested antibiotics, except for colistin (CL), were ineffective against the isolates. The major mechanism of resistance to carbapenems was the coexistence of blaOXA -51-like and ISAba1 genetic elements in 24(100%) isolates, followed by isolates carrying the blaOXA-24-like and blaNDM-1 genes, accounting for 16 (66.7%) and 8 (33.3%) of all isolates respectively. A single effective combination of CL and tigecycline (TGC) exhibited the maximum rate (100%) of synergy against the *A. baumannii* samples. The synergistic effect of CL in combination with TGC was confirmed, which may confer therapeutic benefits against XDR-*A. baumannii*. This finding is valuable and emphasizes the necessity to discover novel combination therapies that are effective against virtually untreatable XDR-*A. baumannii* infections. However, further clinical trials are required to verify the efficacy.

Keywords: synergistic effect; antibiotics; *Acinetobacter baumannii*; CRAB; ISAba1 gene; colistin.

Introduction

The growing problem of multidrug resistance (MDR) among Gram-negative bacterial strains (Radha et al., 2024) and bacteria that are Gram-positive (AL-Salihi et al., 2023; Karim et al., 2019) combined with a slowdown in the discovery of new bioactive compounds, has limited treatment options.

This has led to an increase in mortality rates as we continue to fight and combat infectious diseases, particularly those caused by *A. baumannii* associated organisms within the *A. baumannii–calcoaceticus* complex, which pose a global threat. These bacteria are Gram-negative, belong to the Moraxellaceae family and predominantly cause nosocomial diseases especially in ICUs, such as ventilator-associated pneumonia (VAP), wound infections, urinary tract infections (UTI), meningitis, gastrointestinal and bacteremia (Nasr, 2020). *A. baumannii* ranks high on the WHO target pathogen list as a "critical" threat, highlighting its significance as a nosocomial pathogen, especially when it shows resistance to the antibiotics used as a "last resort". Additionally, carbapenem resistant *A. baumannii* (CRAB) has been categorised as an urgent public health issue by the Center for Disease Control and Prevention (CDC) that reported by (Lodise et al., 2025).

Carbapenem was selected as a marker because CRAB is typically linked to a wide range of co-resistance to different kinds of antibiotics classes (Jesudason, 2024). CRAB is driven by several interacting mechanisms. These include the overexpression of blaOXA genes – either plasmid- or chromosomal-mediated – that encode oxacillinases (such as blaOXA-58-like, blaOXA-51, blaOXA-143, and blaOXA-23). Additionally, the existence of metallo- carbapenemases (such as blaNDM, blaIMP, blaSIM, and blaVIM) along with the ability of porins to inhibit them, which act as pathways for the entrance of carbapenem, contribute to this resistance (Poirel & Nordmann, 2006). *Acinetobacter*'s remarkable genomic flexibility allows fast mutations, rearrangements, and integration of foreign determinants via mobile genetic components. Insertion sequences, such as ISAba1, are key factors that shape bacterial genomes. For instance, they can regulate the expression of OXA-type carbapenemase genes (Ayoub Moubareck & Hammoudi Halat, 2020). CRAB and MDR-*A. baumannii* isolates are found in various locations around the world, including Iraq (Al-Sheboul et al., 2022; Rajan et al., 2023; Al-Khafaji & Al-Fatlawy, 2025; Park et al., 2025).

Treatment of XDR-*A. baumannii* infections is challenging due to the limited antibiotic options available and the fact that these infections often occur in critically ill patients with reduced physiological reserves (Peleg et al., 2008). TGC and CL are effective treatments for XDR-*A. baumannii* infections. Nevertheless, these drugs have a high toxicity level, due to increased resistance (Qureshi et al., 2015). TGC, a semi-synthetic tetracycline product, has demonstrated *in vitro* antibiotic efficacy towards CRAB isolates. However, reports of increasing resistance in various locations, along with instances of TGC monotherapy failure, indicate a need to combine TGC with other antimicrobials (Marchaim et al., 2014). Combination therapy is a promising strategy for optimizing treatment regimens due to microbial resistance mechanisms to achieve better clinical outcomes.

The combination of TGC and CL at medical levels that are clinically relevant effectively treats carbapenem-resistant *K. pneumonia* strains and *A. baumannii*, irrespective of resistance to colistin, as demonstrated by Park et al. (2025). Accordingly, the current study characterized *A. baumannii* isolates, identified their antibiotic susceptibility profiles, the genetic determinant for antibiotic resistance and evaluated the *in vitro* impacts of different combinations of antibiotics towards a group of CRAB clinical isolates.

Materials and methods

In this study, we used bacterial isolates collected from clinical samples from patients, which were delivered to the microbiology laboratory as part of standard routine clinical testing during diagnostic workup at Harem Private Hospital in Sulaymaniyah, Iraq, from September 1st, 2024, to September 31st, 2025.

The clinical samples were inoculated immediately onto different culture media as MacConkey nutrient agar and blood agar, incubated under aerobic conditions for 24 h. at 37 °C (Ness & Olsburg, 2019). The isolated bacterial colonies were subsequently diagnosed with "the BD Phoenix Automated Microbiology System" following the manufacturer's specifications (BD, USA). The bacterial isolates were kept in vials with tryptic soy broth and 15% glycerol, then incubated at 37 ºC for 24 h. under aerobic conditions. Later, they were preserved at –20 ºC until tested according to Bergey's Manual of Determinative Bacteriology (George, 2001).

Minimum inhibitory concentration (MIC) of the antibiotics toward identified isolates was measured utilising the BD Phoenix™ system, USA. The following antibiotics were used ; amikacin (AK), gentamicin (GEN), ertapenem (ETP), imipenem (IMP), meropenem (MER), cefazolin (CZ), cefuroxime (CXM), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), ceftolozane-tazobactam (C/T), ampicillin (AM), amoxicillin-claulanate (AMC), piperacillin-tazobctam (TZP), colistin (CL), trimethoprim-sulfamethoxazole (SXT), nitrofurantoin (F), ciprofloxacin (CIP), levofloxacin (LEV), and tigecycline (TGC). The Clinical and Laboratory Standards Institute (CLSI) breakpoints for assessing sensitivity were used to interpret the data; sensitivity, resistance, or intermediate status (CLSI, 2024). However, there is not a known TGC breakpoint in *Acinetobacter* species, so we used the most recent FDA-Identified Interpretive Criteria for Enterobacteriaceae to assess the TGC MIC breakpoints. According to these criteria, an MIC of ≥ 8 mg/L is considered as resistant, 4 mg/L as intermediate, and ≤ 2 mg/L is as sensitive. The MIC for CL was measured using Broth Micro-Dilution (BMD), with CL sensitivity graded as resistant (MIC ≥ 4 μg/mL) or sensitive (MIC ≤ 2 μg/mL) (CLSI, 2014).

Chequerboard tests were conducted as reported by Deolankar et al.(2022). In a 96-well plate, two kinds of antibiotics were serially diluted (two fold) in opposite vertical and horizontal directions. Diluted bacterial cells were added at OD_{600} = 0.05, and following overnight incubation, the OD_{600} was read out. The combination effect for each antibiotic was determined by calculating the FICI, that is the MIC of the antibiotic agent in combined form and dividing on the MIC of the antibiotic agent alone.

PureLink® Genomic DNA Kit was used to extract genomic DNA from bacterial culture samples (Thermo Fisher, USA) in compliance with the manufacturer's specification, and was later used for the PCR analyses using Bio-Rad C1000 Touch Thermal Cycler + CFX96 Real Time System Lab (BIORAD, USA). Each PCR reaction was run in 25 μL, as a final volume, which contained 2 μL of the extracted DNA, the required concentrations of primers, and 1X AddStart Taq Master Ready Mix (ADDBIO INC., Korea). The following genes (16s rRNA, blaOXA-58-like, blaOXA-51-like, blaOXA-24-like, and blaOXA-23-like) were screened, applying a previously published multiplex PCR assay (Woodford et al., 2006), and the isolates were also screened for blaIMP-1, blaSIM-1 and blaNDM-1 and ISba1 insertion sequence genes, Table 1. The Nanodrop Lite spectrophotometer (ThermoFisher Scientific, USA) was used to measure DNA concentrations, which ranged from 50 to 120 ng/μL with absorbance 260–280 ratios between 1.91 and 2.0. The proliferated DNA sequences with PCR were analysed employing 1.5% agarose in horizontal electrophoresis and a 100bp DNA ladder (Genes and Biotech Co. Ltd, China) was used as a standard molecular marker. The PCR products were visualized by UV light at 366 nm and photographed with a digital camera.

Table 1
The primers used for screening antibiotic resistant genes

Primers	Sequence (5'-3')	Target gene	Base pair	Annealing temperature, °C	Reference
IS*Aba1*-F IS*Aba1*-R	CACGAATGCAGAAGTTG CGACGAATACTATGACAC	IS*AbaI*	549	56	Segal et al. (2005)
OXA-58-likeF OXA-58-likeR	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	*blaOXA-58-like*	599	52	Woodford et al. (2006)
OXA-51-likeF OXA-51-likeR	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	*bla*OXA-51-like	353	52	
OXA-24-likeF OXA-24-likeR	GGTTAGTTGGCCCCCTTAAA AGTTGAGCGAAAAGGGGATT	*bla*OXA-24-like	246	52	
OXA-23-likeF OXA-23-likeR	ATCGGATTGGAGAACCAGA ATTTCTGACCGCATTTCCAT	*bla*OXA-23-like	501	52	
NDM-1 F R	CAATATTATGCACCCGGTCG CCTTGCTGTCCTTGATCAGG	*bla*INDM-1	632	52	Kaase (2012)
*bla*IMP-1 F R	CATGGTTTGGTGGTTCTTGT ATAATTTGGCGGACTTTGGC	*bla*IMP-1	188	52	Ellington et al. (2007)
SIM-1F R	TAC AAGGGATTCGGCATCG TAATGGCCTGTTCCCATGTG	*bla*ISIM-1	570	54	
16SrRNA 8F 1541R	AGAGTTTGATCCTGGCTCAG AAGGAGGTGATCCAGCCGCA	16S rRNA	1500	58	Unnikrishnan et al. (2018)

The descriptive statistical analysis that incorporates "percentages and frequencies" was measured for the variables in question. The FICI was determined using the formula: FICI = (MIC AA+B/MIC A) + (MIC BA+B/MIC B). Here, MIC A and MIC B represent the minimum inhibitory concentration of each antibiotic agent when used individually, while MIC AA+B and MIC BA+B represent the minimum inhibitory concentrations of the antibiotics used in combination. A FICI value of less than 0.5 exhibits a synergistic action of the antibiotic combination, while a FICI between 0.5 and 1.0 suggests additive effects. A FICI ranging from 1 to 4 indicates an indifferent combinatorial effect, whereas a FICI greater than 4 signifies an antagonistic interaction.

Results

Twenty four isolates of *A. baumannii* were collected from the ICU for this investigation. The majority of them (20) were isolated from sputum samples, and four samples of bronchial wash, accounting for 83.3%, and 16.7% of the total samples, respectively (Fig. 1).

All isolates were resistant to a panel of 19 antibiotics including AK, GEN, ETP, IMP, MER, CZ, CXM, CAZ, CRO, FEP, C/T, AM, AMC, TZP, SXT, F, CIP, LEV, and TGC with MIC values of (>32, >8,>4, >4, >16, >16, >16, >32, >16, >8/4, >16, >64/4, >4/76, >64, >2, >4, and >4) respectively. Whereas, CL susceptibility couldn't be detected by the automated system, it exhibited "susceptible" values against all strains under investigation which were measured by the BMD test. Overall, this sample of *A. baumannii* bacteria was highly MDR, including last-resort medications and standard of care for *A. baumannii* infections. Furthermore, all of them (100%) were found to be CRAB, and XDR-*A. baumannii* strains.

The investigated isolates were examined for acquired antibiotic resistance determinants using singleplex and multiplex PCR tests, which included blaOXA-58-like, blaOXA -51-like, blaOXA-24-like,

blaOXA-23-like, blaIMP-1, blaSIM-1, blaNDM-1, and ISAbaI genes as shown in (Table 2, Fig. 1). The primary cause of resistance to carbapenem was the concurrent existence of blaOXA-51-like, and ISAbaI elements 24 (100%) in all studied isolates, followed by harbouring of the blaOXA-24-like and blaNDM-1 gene, accounting for 16 (66.7%) and 8 (33.3%), respectively. However,, none of the isolates were found to carry blaOXA-58-like, blaOXA-23-like, blaIMP-1, and blaSIM-1 genes.

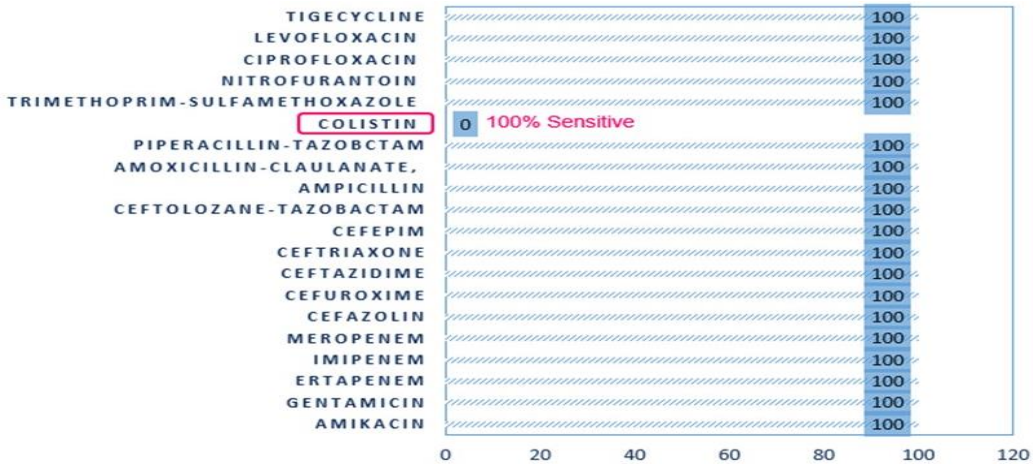

Fig. 1. Antibiotic sensitivity pattern of *A. baumannii* strains (N = 24)

The Σ FIC for the four CL based antibiotics combination CL + TGC, CL + IMP, CL + MER and CL + LEV and their effects (synergism, additive, and indifferent) were calculated against all studied isolates as shown in Figure 2. A single highly effective combination CL + TGC exhibited the maximum rate of synergy, 24 (100%) against the bacterial isolates, followed by CL + LEV 2 (8.3%). Whereas CL + MER demonstrated the highest 24 (100.0%) indifferent effect, followed by CL + LEV, and CL + IMP accounting for 20 (83.3%) and 12 (50.0%) of the total samples, respectively. Additionally, the additive effect of CL-based combinations with either IMP or LEV was observed in 12 (50.0%), and 2 (8.3%) of the studied isolates, respectively.

Table 2
Frequency of antibiotic-resistant genes in the studied isolates

	Genes	Number	%
1	IS*AbaI*	24	100.0
2	*bla*OXA-58-like	0	0.0
3	*bla*OXA -51-like	24	100.0
4	*bla*OXA-24-like	16	66.7
5	*bla*OXA -23-like	0	0.0
6	*bla*IMP-1	0	0.0
7	*bla*SIM-1	0	0.0
8	*bla*NDM-1	8	33.3

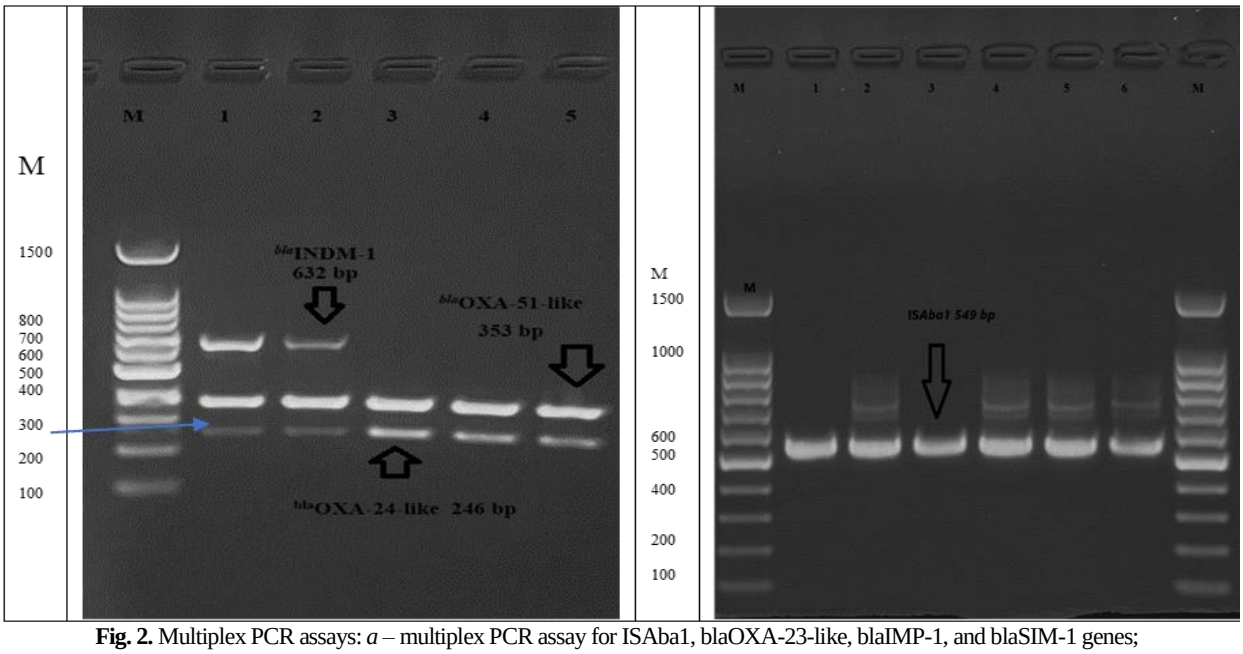

Fig. 2. Multiplex PCR assays: *a* – multiplex PCR assay for ISAba1, blaOXA-23-like, blaIMP-1, and blaSIM-1 genes; multiplex A 1.5% agarose gel was utilized to separate the PCR proliferated products: lanes 1 – 100 bp molecular marker (M), lanes 1–5 – *A. baumannii* No. 1–5; *b* – multiplex PCR assay for the blaOXA-58-like, blaOXA-51-like, blaOXA-24-like, and blaNDM-1 genes; lanes M – 100 bp molecular marker, 1–6 – *A. baumannii* No. 1–6

Discussion

Considering the growing risk of antimicrobial resistance among *Acinetobacter* species, it is essential to identify effective antimicrobial agents, whether as standalone treatments or in combination regimens for CRAB and XDR-*A. baumannii* strains infections. We analysed in the present study the pattern of sensitivity to antibiotics of 24 clinically isolated strains of extremely antibiotic resistant *A. baumannii*. All bacterial strains isolated from sputum and bronchial wash samples exhibited 100% phenotypic resistance to carbapenems, as determined by MIC testing of imipenem, ertapenem, and meropenem. These strains were also classified as XDR, they were non-sensitive to ≥1 agent in all but ≤2 antibiotic classes using the selection criteria stated by (Magiorakos et al., 2012).

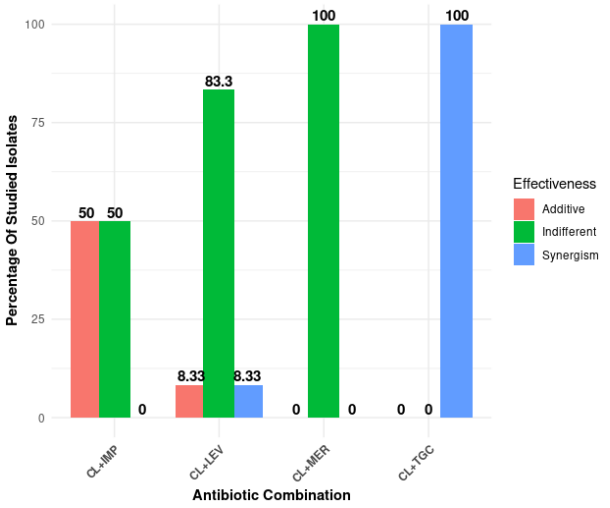

Fig. 3. Effect of different antibiotics in combination on the studied isolates

The resistance rate exhibited by this collection is clearly higher than that reported in another recent study in Sulaimanyah, Iraq, which found MDR (54.3%), XDR (39.1%), PDR (6.5%), and up to 93.4% CRAB among 46 *A. baumannii* isolates from different medical samples (Muhammed & Rasool, 2025). Additionally, other studies in Erbil and Duhok, Iraq, showed resistance of isolates to the most available antibiotics, including carbapenems (Qader & Ganjo, 2023). High rates of resistance to antibiotics are often linked to numerous considerations notably the irrational use and overuse of broad-spectrum antibiotics in ICUs, poor hygiene practices, self-medication and easy access to antibiotics, incomplete treatment courses, and the use of these medications in agriculture and livestock rearing (Kempf & Rolain, 2012; Kurdi et al., 2024; Darweesh et al., 2025; Saleem et al., 2025). Additionally, prolonged stays in the ICU and invasive procedures – such as tracheostomy, urinary catheterization, and vascular catheterization – often lead to conditions such as pneumonia, bacteraemia and UTI (Kuo et al., 2007).

Based on multiplex PCR, plex PCR, the studied bacterial isolates were screened for the following genes which confer resistance to carbapenems: blaOXA-58-like, blaOXA-51-like, blaOXA-24-like, blaOXA-23-like, blaIMP-1, blaSIM-1, blaNDM-1, and ISAba1. The primary cause of carbapenem resistance was combination of blaOXA-51-like and ISAba1 sequence, accounting for 24 (100%) isolates, followed by isolates harbouring blaOXA-24-like and blaNDM-1 genes, accounting for 16 (66.7%) and 8 (33.3%) of isolates, respectively. However, none of the studied isolates were identified to harbour blaOXA-58-like, blaOXA-23-like, blaIMP-1 and blaSIM-1.

Similar to our result, a local study reported on 120 *A. baumannii-calcoaceticus* complex isolates from clinical samples in Sulaimanyah and Erbil hospitals, Iraq. All their isolates carried the blaOXA-51-like gene, and were negative for the blaOXA-58-like gene in the 110 imipenem-resistant strains. In contrast to our findings, 92% of their isolates carried the blaOXA-23 gene, and only 3% tested positive for blaOXA-24-like (Ganjo et al., 2016). An additional study in Baghdad, Iraq, reported most of their clinical *A. baumannii* isolates as carrying blaOXA-51-like, and ISAba1 element, whereas they lacked the blaOXA-58-like gene (Al-Masoudi et al., 2015). Furthermore, other investigators in Asia (Wong et al., 2019) verified that carbapenem resistance in *A. baumannii* is mostly caused by excessive production of carbapenem-hydrolyzing-class D-ß-lactamases, such as the blaOXA-23-like, and blaOXA-24-like and blaOXA-51-like genes.

It is crucial to emphasize that the production of OXA-type carbapenemase enzymes in *A. baumannii* are heavily regulated by correlated mobile genetic sequences, such as ISAba1 insertion sequence, which provides a potent promoter, and leads to higher resistance to carbapenem. One such instance is blaOXA-51, which is inherent to *A. baumannii* and is commonly employed as a marker to identify this species of bacteria. However, it has poor carbapenemase efficacy. But as, connected with ISAba1 upstream, its production and expression are greatly increased, conferring significant resistance to carbapenem treatment (Evans & Amyes, 2014; Al-Sheboul et al., 2022).

Furthermore, IS elements promote the spread of blaOXA variations between strains and geographical areas worldwide including Iraq (Al-Sheboul et al., 2022; Rajan et al., 2023) by facilitating horizontal gene transfer, which makes infection management and treatment measures even more challenging (Evans & Amyes, 2014).

The presence of blaNDM-1, which belongs to the metallo-β-lactamase gene, at 33.3% in the current study raises various questions. Isolates harboring this gene have been recorded from many countries (Abduljabar & Mawlood, 2023; Rajan et al., 2023; Al-Khafaji & AL-Fatlawy, 2025). A recent investigation revealed that the worldwide spread of this gene is mostly attributed to transposon jumps and horizontal transfers of plasmids among microbial cells having a significant impact on regional transmission (Acman et al., 2022). It has been shown in the current study that each isolate possesses at least three of the genes under investigation. This is in agreement with other studies (Al-Sheboul et al., 2022; Rajan et al., 2023; Al-Khafaji & Al-Fatlawy, 2025). This fact suggests that an increase in their combined activity, could result in a rise in resistance to antibiotics among bacterial species (Shi et al., 2024).

The findings of the current study show that antibiotics typically used as last-resort therapies for multidrug-resistant (MDR) species show alarmingly elevated levels of resistance, including carbapenems and tigecycline (TGC). These resistant strains are often nearly untreatable and associated with high fatality rates. A combination of therapies might be the best effective treatment strategy for rapidly discovering innovative therapeutic approaches, especially given the slow introduction of new drugs into the market. Therefore, we tested four combinations of CL-based antibiotics against our entire collection to determine potential synergistic impacts, as shown in Figure 3.

CL was the most successful of the tested last-resort antibiotics, with 100% of strains showing susceptibility in the current study. CL remains the most highly effective antibiotic for treating *A. baumannii* infections when compared to other tested antibiotics. This result is in line with earlier investigators who reported lack of resistance to CL among their clinical *A. baumannii* isolates (Mohammed et al., 2021). Moreover, other researchers in Sulaimanya, Iraqi, found the isolates' lowest resistance rate (6.5%) was against CL (Muhammed & Rasool, 2025). Although CL displays efficiency against XDR-*A. baumannii* strains *in vitro*, its clinical application in treating ventilator-associated pneumonia (VAP) has been challenged due to poor penetration into the lung tissue. As a result, CL was used in association with other antibiotics, most frequently with carbapenem or rifampin (Perez et al., 2007). The combination of CL and TGC, rather than using TGC alone, is now essential for reducing clinical failure in critically ill patients. The effectiveness of TGC as a monotherapy is often challenged because of its poor concentration in serum and restricted diffusion into the epithelial lining fluid (Karaiskos et al., 2019).

TGC-CL combination showed a synergistic effect in all of the tested isolates in our study while CL-IPM, CL -MER, and CL-LEV showed indifferent results, followed by the additive effect in the majority of the tested isolates. Nevertheless, no antagonistic interaction was identified in any of the drug combinations. Supporting the findings of our study, CL showed a comparable synergistic reaction with TGC against CRAB and *K. pneumoniae* (Pournaras et al., 2011; Karaoglan et al., 2013; Ku et al., 2017; Park et al., 2025). Thereafter, TGC and CL at clinically feasible concentrations showed synergistic efficacy *in vitro* and *in vivo* against *E. coli*, and the murine thigh model respectively (Zhou, 2020). Furthermore, a previous study confirmed that the combination of TGC-CL showed synergistic, additive and indifferent effect in 10.0% 23.3% and 66.7% of isolates respectively (Mohammed et al., 2021). While CL is bactericidal, TGC has bacteriostatic properties. It suppresses protein synthesis mechanism in bacteria, consequently altering their cells. Subsequently, CL may then cause bacterial cells to die by rupturing their plasma membrane. CL can enhance the effectiveness of other antibiotics by disrupting cell walls and membranes (Park et al., 2025). Furthermore, due to the risk of CL resistance, it should only be used for serious infections brought on by MDR *A. baumannii*. Hence, various treatment approaches for

treating VAP brought on by *A. baumannii*, must be considered (Cikman, 2015).

Conclusion

This investigation verified the synergistic impact of CL when combined with TGC. In addition, CL-based combinations with either MER or LEV may confer therapeutic benefits against CRAB and XDR *A. baumannii*. However, further clinical trials are required to prove efficacy. These findings are valuable and emphasize the necessity to discover novel combination therapies that are effective against virtually untreatable CRAB and XDR-*A. baumannii* infections.

The current investigation received permission from the Scientific and Ethics Committee of the Nursing College at the University of Kirkuk, Iraq, under reference number 10 on May 8, 2025. Additionally, official permission was granted by Harem Private Hospital in Sulaymaniyah City, Iraq, with reference number 757 on June 4, 2025. No experiments involving humans or animals are described in this publication.

The authors declare, the research contains no conflicts of interest.

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