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Impact of blaOXA and blaNDM production on the efficacy of colistin-based antibiotic combinations in carbapenem-resistant Acinetobacter baumannii from a tertiary care hospital in North Kerala, India

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Research Article

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Abstract

Background

Acinetobacter baumannii is a major cause of healthcare-associated infections and is increasingly recognised for its ability to develop multidrug resistance, particularly to carbapenems. The emergence of carbapenem-resistant *A. baumannii* (CRAB) poses significant challenges to treatment and infection control in high-burden regions such as India. This study aimed to characterise the phenotypic resistance patterns, molecular determinants of carbapenem resistance, and *in vitro* effectiveness of selected combination drug therapies among clinical isolates of CRAB from a tertiary care hospital in North Kerala.

Methods

The study was conducted at the Malabar Institute of Medical Sciences (MIMS), Kozhikode, North Kerala, from January 2024 to December 2025. The ethical clearance for the present study was sought from the Institutional Ethics Committee (IEC) with Study No. EC/112/24. A total of 199 non-duplicate *Acinetobacter* isolates from diverse clinical specimens were analysed. Antimicrobial susceptibility testing was performed using standard methods, and carbapenem-resistant isolates were subjected to CarbAcineto NP testing for phenotypic confirmation. Molecular characterisation targeted key carbapenemase genes (bla_{OXA-51}, bla_{OXA-23}, and bla_{NDM}). Fifty phenotypically confirmed CRAB isolates underwent *in vitro* combination drug testing (colistin + meropenem; colistin + tigecycline) using the Epsilometer (E-test) method, with interactions assessed using the Fractional Inhibitory Concentration Index (FICI).

Results

Respiratory samples contributed the highest proportion of isolates (32.7%), followed by urine and tissue/pus specimens. Carbapenem resistance was detected in 40.2% (n = 80) of isolates, of which 87.5% were CarbAcineto-NP-positive. Genotypically, bla_{OXA-51} + bla_{OXA-23} was the most prevalent profile, (n = 52), followed by isolates carrying bla_{NDM} either alone with bla_{OXA-51} (n = 25) or in combination with bla_{OXA-23} (n = 25). *In vitro* synergy was not observed with colistin + meropenem, which exhibited predominant antagonism (60%). Colistin + tigecycline showed synergy in 20% of isolates, with the remainder demonstrating indifference or antagonism.

Conclusion

CRAB isolates in this North Kerala tertiary-care setting demonstrated high levels of multidrug resistance and substantial carbapenemase diversity dominated by OXA- and NDM-type genes. Colistin–tigecycline exhibits limited but consistent synergy, whereas colistin–meropenem lacks efficacy and frequently shows antagonistic interactions in CRAB isolates. Molecular determinants such as bla_{OXA-23} and bla_{NDM} do not significantly predict combination therapy outcomes, highlighting the need for phenotypic-guided treatment strategies.

Introduction

Acinetobacter baumannii has become one of the most formidable multidrug-resistant (MDR) pathogens encountered in modern healthcare systems, particularly within intensive-care and high-dependency units. Its extraordinary ability to withstand desiccation, persist on hospital surfaces, and colonise vulnerable patients has enabled its rapid global spread and has cemented its role as a critical cause of healthcare-associated infections [1, 6]. The organism is implicated in diverse and severe clinical manifestations, including ventilator-associated pneumonia, bloodstream infections, meningitis, urinary tract infections, and wound infections—conditions that remain especially prevalent among critically ill and immunocompromised individuals [2]. The global emergence of carbapenem-resistant *A. baumannii* (CRAB) has further heightened public health concern, owing to severely limited therapeutic options and the organism's capacity to acquire resistance determinants at exceptional speed [6]. Carbapenem resistance is driven largely by carbapenemase enzymes—particularly OXA-type β-lactamases such as bla_{OXA-23} (β-lactamases OXA-23) that are frequently associated with successful hospital-adapted clones and have facilitated widespread international dissemination [1]. Increasingly,

hospital outbreaks and regional clusters have been documented across healthcare networks, highlighting ongoing challenges related to infection control, antimicrobial pressure, and inter-facility transmission [3].

Within India, CRAB poses a growing threat, exacerbated by high antibiotic consumption, overburdened healthcare settings, and the organism's ability to form robust biofilms that enhance survival and antimicrobial resistance [19]. These factors contribute to persistent contamination and rising resistance levels, as well as the presence of multiple carbapenemase determinants in both clinical and environmental isolates [4]. Understanding the phenotypic resistance characteristics and genotypic carbapenemase profiles within this regional context is essential for guiding treatment algorithms, informing antimicrobial stewardship, and shaping infection prevention strategies. As therapeutic options narrow, combination drug therapy has gained importance as a proposed strategy to improve outcomes in infections caused by CRAB. However, global evidence demonstrates marked variability in synergistic, indifferent, or antagonistic effects between antimicrobial combinations—particularly regimens involving polymyxins, tigecycline, and carbapenems—underscoring the need for localised, isolate-specific evaluation to inform clinical practice. [1, 3]

Against this background, the present study aims to comprehensively characterise the phenotypic antibiotic resistance patterns, molecular carbapenemase gene profiles, and *in vitro* effectiveness of combination drug therapy among clinical isolates of carbapenem-resistant *Acinetobacter baumannii* obtained from a tertiary care hospital in North Kerala, India. This integrated approach is intended to support improved therapeutic decision-making and strengthen local antimicrobial stewardship efforts.

Materials and Methods

Study Design and Setting

This was a prospective observational laboratory-based study conducted in the Department of Microbiology of a tertiary care hospital in North Kerala, India, from January 2024 to December 2025. The ethical clearance for the present study was sought from the Institutional Ethics Committee (IEC) at Malabar Institute of Medical Sciences (MIMS), Kozhikode, North Kerala, India, with Study No. EC/112/24.

Sample Size and Clinical Specimens

The sample size was calculated using the single population proportion formula:

$n = Z^2 \times P \times (1 - P)/d^2$

Where:

- **n** = required sample size
- **Z** = Z statistic at 95% confidence level (1.96)
- **P** = expected prevalence
- **d** = absolute precision (margin of error)

Previous studies from India have reported carbapenem resistance rates among *Acinetobacter baumannii* ranging from 40% to 75% [14]. Based on these reports and considering a prevalence of carbapenem-resistant *Acinetobacter* species of 40.2%, observed among *Acinetobacter* isolates in similar healthcare settings, a 95% confidence level (Z = 1.96), and a precision of 7%. The calculated minimum sample size was 189 isolates. After accounting for a 5% contingency for potential sample loss and incomplete data, the final sample size was estimated to be 199 isolates. Therefore, a total of 199 non-duplicate clinical isolates of *Acinetobacter* spp. were obtained from various clinical specimens, including respiratory samples (bronchial wash, sputum, tracheal aspirate, and mini bronchoalveolar lavage); urine; tissue and pus; blood; vaginal swabs; and sterile body fluids (ascitic fluid, breast milk, and pleural fluid). Only the first isolate per patient was included to avoid duplication. The inclusion criteria involved laboratory-confirmed *Acinetobacter* spp. isolates from the samples of all age groups, while the exclusion criteria were the following: 1) Organisms other than *Acinetobacter spp.* 2) actively contaminated or mixed growth is excluded.

Isolation and Identification of Acinetobacter spp.

Clinical specimens were processed according to standard microbiological procedures. Samples were cultured on blood agar and MacConkey agar and incubated aerobically at 37°C for 18–24 hours. Non-lactose-fermenting colonies with characteristic morphology were subjected to preliminary identification using Gram staining, the oxidase test, and biochemical tests. Species-level identification was confirmed using standard identification systems following Clinical and Laboratory Standards Institute (CLSI) [18] guidelines. *Acinetobacter baumannii* ATCC 19606 was used as the positive reference control strain, while *Escherichia coli* ATCC 25922 served as a negative and quality control strain for phenotypic and biochemical identification procedures.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar as per CLSI guidelines [18]. The antibiotics tested included amikacin (AK, 30 µg), cefepime (FEP, 30 µg), cefoperazone–sulbactam (CFS, 75/30 µg), ciprofloxacin (CIP, 5 µg), cotrimoxazole (COT, 1.25/23.75 µg), gentamicin (GEN, 10 µg), imipenem (IPM, 10 µg), meropenem (MEM, 10 µg), piperacillin–tazobactam (TZP, 100/10 µg), minocycline (MIN, 30 µg), and levofloxacin (LEV, 5 µg). *Escherichia coli* ATCC 25922 and *Acinetobacter baumannii* ATCC 19606 was used as a quality-control strain to validate disc potency and testing conditions. Results were interpreted as susceptible, intermediate, or resistant according to CLSI breakpoints.

Screening and Phenotypic Confirmation of Carbapenem Resistance

Isolates showing resistance to imipenem and/or meropenem were categorised as carbapenem-resistant. These isolates were further subjected to phenotypic confirmation of carbapenemase production using the CarbAcineto NP test [14]. *Acinetobacter baumannii* ATCC BAA-1705 (carbapenemase-producing) and *A. baumannii* ATCC BAA-1706 (non-carbapenemase-producing) were used as positive and negative control strains, respectively. A colour change indicating carbapenem hydrolysis was interpreted as a positive result. Only phenotypically confirmed carbapenem-resistant isolates were included for molecular analysis.

Molecular Detection of Carbapenem Resistance Genes

Genomic DNA Extraction

Genomic DNA from the bacterial isolates was extracted using the HiMedia HiPurA Bacterial Genomic DNA Purification Kit from HiMedia Laboratories, India, according to the manufacturer's instructions. Briefly, bacterial cells were lysed, and DNA was then purified on silica spin columns by sequential washing and elution steps according to the kit protocol. The extracted DNA was stored at -20°C until use as a template in PCR amplification of target genes.

Polymerase Chain Reaction (PCR)

Molecular confirmation for *Acinetobacter spp.* was performed by detection of the recA gene [27], as shown in Table 1. Amplification of $bla_{OXA-51-like}$, $bla_{OXA-23-like}$, and bla_{NDM} genes was performed by PCR in a total reaction volume of 50 µL containing Taq PCR master mix, gene-specific primers, template DNA, and nuclease-free water. For the bla_{NDM} gene, amplification was done by denaturation at 94°C for 10 min, followed by 36 cycles of 94°C for 30 s, annealing at 52°C for 40 s, and extension at 72°C for 50 s, followed by a final extension at 72°C for 5 min [12, 26, 27]. Amplification of $bla_{OXA-51-like}$ and $bla_{OXA-23-like}$ genes was carried out for 35 cycles, each consisting of denaturation for 45 s at 94°C, annealing for 45 s at 57°C and extension at 72°C for 1 min. Initial denaturation was carried out at 94°C for 3 min, while final extension was done at 72°C for 5 min [17]. Agarose gel electrophoresis with 1x TAE buffer, ethidium bromide staining, and visualisation under a UV transilluminator were employed to analyse the PCR products. The presence of the specific amplicon bands indicated the detection of carbapenem-resistance genes. [13]

Table 1
Lists the sequences of the primers used

PRIMER	Sequence (5' to 3')	Target	Base-pair	References
NDM-Forward primer	GGTTTGGCGATCTGGTTTTC	*bla* $_{NDM}$	621	[26, 27]
NDM-Reverse primer	CGGAATGGCTCATCACGATC			
OXA-23-like Forward primer	GATCGGATTGGAGAACCAGA	*bla* $_{OXA-23}$	501	[17, 27]
OXA-23-like Reverse primer	ATTTCTGACCGCATTTCCAT			
OXA-51-like Forward primer	TAATGCTTTATCGGCCTTG	*bla* $_{OXA-51}$	353	[17, 27]
OXA-51-like Reverse primer	TGGATTGCACTTCATCTTGG			
recA-Forward primer	CCTGAATCTTCTGGTAAAAC	*recA*	425	[27]
recA-Reverse primer	GTTTCTGGGCTGCCAAACATTAC			

In Vitro Combination Drug Testing

Selection of Isolates

Out of the phenotypically and genotypically confirmed carbapenem-resistant *Acinetobacter* spp., 50 isolates were selected for *in vitro* combination drug testing.

Epsilometer (E-test) Method and Interpretation of drug interactions

Combination testing was performed using the Epsilometer (E-test) method [15]. Antibiotic combinations tested included colistin + meropenem and colistin + tigecycline [14, 20, 21, 22]. MICs of individual antibiotics and their combinations were determined on Mueller–Hinton agar. *Escherichia coli* ATCC 25922 and *Acinetobacterbaumannii* ATCC 19606 was used as a quality-control strain to ensure assay validity and MIC accuracy. Drug interactions were interpreted using the Fractional Inhibitory Concentration Index (FICI) calculated as MIC FICI = MIC of drug A alone/MIC of drug A in combination + MIC of drug B alone/MIC of drug B in combination. The interactions were categorised as synergy (FICI ≤ 0.5), indifference (FICI > 0.5 to ≤ 4.0) and antagonism (FICI > 4.0)

Statistical Analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) software version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarise categorical variables as frequencies and percentages. Associations between categorical variables, including specimen type, antimicrobial resistance pattern, carbapenemase gene profile, and combination therapy outcomes, were assessed using the Pearson chi-square test. When the assumptions of the chi-square test were violated (i.e., expected cell frequencies < 5 or the presence of zero cell counts), the Fisher–Freeman–Halton exact test was applied. A p-value < 0.05 was considered statistically significant.

Results

Clinical and Demographic Distribution of Acinetobacter spp.

A total of 199 non-duplicate *Acinetobacter* spp. isolates were obtained from various clinical specimens. Respiratory samples constituted the highest proportion (32.7%, n = 65), followed by urine (29.6%, n = 59) and tissue/pus samples (23.6%, n = 47). Blood samples accounted for 10.1% (n = 20), while vaginal swabs (2.5%, n = 5) and body fluids (1.5%, n = 3) contributed minimally. Among the patients, 53.27% (n = 106) were males and 46.73% (n = 93) were females, with a male-to-female ratio of 1.14:1. The mean age of male patients was 53.76 years, while that of female patients was 48.98 years, indicating a higher prevalence among older individuals (Fig. 1) .

Antimicrobial Resistance Pattern

Antimicrobial susceptibility testing revealed high resistance rates across multiple antibiotic classes. Resistance was most frequently observed against ciprofloxacin (44.2%), cefepime (43.7%), and piperacillin–tazobactam (41.1%). Resistance to aminoglycosides and β-lactam/β-lactamase inhibitor combinations ranged between 33–41%. Carbapenem resistance to both imipenem and meropenem was detected in 40.2% (n = 80) of isolates, indicating a substantial burden of multidrug resistance. Minocycline showed the lowest resistance rate (20.1%), suggesting retained activity **(Fig. 2)**.

Figure 2 Antimicrobial susceptibility patterns and resistance profile of clinical Acinetobacter spp. isolates. a–f. Representative Kirby–Bauer disc diffusion assays performed on Mueller–Hinton agar showing the antimicrobial susceptibility patterns of *Acinetobacter* spp. isolates. **g.** Overall antimicrobial resistance rates of *Acinetobacter* spp. isolates against the selected antimicrobial agents included in the study.

Phenotypic Confirmation of Carbapenem Resistance

All 80 carbapenem-resistant isolates were subjected to phenotypic confirmation using the CarbAcineto NP test. Of these, 70 isolates (87.5%) tested positive, while 10 (12.5%) were negative. Only the 70 phenotypically confirmed carbapenem-resistant isolates were selected for molecular analysis and combination drug testing (Fig. 3**)**.

Molecular Detection of Carbapenem Resistance Genes

PCR-based genotyping revealed the widespread presence of OXA-type carbapenemase genes, either alone or in combination with metallo-β-lactamase genes. The *recA* gene produced the expected 425 bp amplicon, confirming the identity of the isolates **(Fig. 4b)**. The intrinsic bla_{OXA-51} gene (353 bp) and the acquired bla_{OXA-23} gene (501 bp) were successfully amplified in representative carbapenem-resistant isolates **(Fig. 4a)**. The bla_{NDM} gene was detected as a 621 bp amplicon in representative positive isolates **(Fig. 4c)**. The distribution of carbapenem resistance genes **(Fig. 4d)** showed that bla_{OXA-51} + bla_{OXA-23} was the predominant genotype, followed by bla_{OXA-51} + bla_{OXA-23} + bla_{NDM} and bla_{OXA-51} + bla_{NDM}, while bla_{OXA-51} alone was detected in a smaller proportion of isolates. The most common genetic profile was bla_{OXA-51} + bla_{OXA-23}, detected in 52 isolates. The NDM gene was detected in 25 isolates in combination with bla_{OXA-51} and in 25 isolates along with both bla_{OXA-51} and bla_{OXA-23}. A smaller subset (17 isolates) carried bla_{OXA-51} alone. These findings indicate that OXA-type carbapenemases, particularly bla_{OXA-23}, were the major molecular determinants of carbapenem resistance among the study isolates.

Figure 4 PCR amplification of species-specific (*recA*) and carbapenem resistance genes ($bla_{OXA-51-like}$, $bla_{OXA-23-like}$, and bla_{NDM}) in *Acinetobacter* spp. and distribution of carbapenem resistance genes. **a.** Amplification of bla_{OXA-51} gene (353 bp). Lane 1: 100 bp DNA ladder; Lanes 2–6, 5: Positive samples showing bands at 353 bp and amplification of bla_{OXA-23} gene (501 bp). Lane 1: 100 bp DNA ladder; Lanes 2–6: 5 positive samples showing bands at 501 bp. **b**. showing amplification of the *recA gene* (425 bp) for confirmation of *Acinetobacter spp.* Lane 1: 100 bp DNA ladder; Lanes 3–7: 5 Positive samples showing distinct bands at 425 bp. **c**. amplification of bla_{NDM} gene (621 bp). Lane 1: 100 bp DNA ladder; Lanes 3, 5–7: 4 Positive samples showing bands at 621 bp. **d** Distribution of carbapenem resistance genes.

In Vitro Combination Drug Therapy

Out of the 70 molecularly confirmed carbapenem-resistant isolates, 50 isolates were randomly selected for *in vitro* combination drug testing using the Epsilometer (E-test) method. Drug interactions were interpreted using the Fractional Inhibitory Concentration Index (FICI). Among the tested combinations, colistin + tigecycline demonstrated better *in vitro* activity compared to colistin + meropenem. Synergistic interaction was observed in 10 isolates with colistin + tigecycline, whereas 20 isolates each showed indifferent and antagonistic interactions. In contrast, colistin + meropenem showed no synergistic activity, with indifferent and antagonistic interactions observed in 20 and 30 isolates, respectively. The difference in interaction patterns between the two combinations was statistically significant ($\chi^2 = 12.0$, $p = 0.002$). Among the isolates exhibiting synergistic interactions with the colistin–tigecycline combination, the MIC of colistin decreased from 2–4 µg/mL when tested alone to 0.25–0.5 µg/mL in combination **(Fig. 5a**, Table 2**)**. Similarly, the MIC of tigecycline was reduced from 1–2 µg/mL to 0.064–0.125 µg/mL, indicating enhanced antibacterial activity of the combination therapy **(Fig. 5b**, Table 2**)**. In isolates demonstrating antagonistic interactions, the MIC of colistin increased substantially from 0.5–2 µg/mL to 16–64 µg/mL following combination with either tigecycline or meropenem, whereas the MICs of the partner antibiotics remained largely unchanged **(Fig. 5c, d;** Table 2**).** This increase in colistin MIC indicated a marked reduction in antibacterial efficacy during combination therapy. For isolates exhibiting indifferent interactions, the MIC values of both antibiotics remained essentially unchanged after combination therapy. Colistin MICs generally ranged between 1 and 2 µg/mL, while meropenem or tigecycline MICs also remained comparable to those obtained with the individual agents, indicating neither

enhancement nor reduction in antimicrobial activity. **(Fig. 5e, f;** Table 2**).** These findings suggest that colistin + tigecycline may provide superior therapeutic potential against carbapenem-resistant *Acinetobacter spp*. compared to colistin + meropenem.
Figure 5 (a–f). Representative E-test (Epsilometer) results illustrating the *in vitro* interactions of colistin–meropenem and colistin–tigecycline combinations against carbapenem-resistant *Acinetobacter* spp. isolates, showing synergistic **(a, b)**, indifferent **(c, f)**, and antagonistic effects **(d, e)**.

Table 2
In vitro interaction of colistin-based antibiotic combinations against 50 carbapenem-resistant *Acinetobacter* spp. (CRAB) isolates determined by the E-test FIC method, with representative changes in minimum inhibitory concentrations (MICs).

Drug Combination	Synergy n (%)	Representative MIC Changes (Synergy)	Indifference n (%)	Representative MIC Changes (Indifference)	Antagonism n (%)	Representative MIC Changes (Antagonism)	χ^2 value	p-value
Colistin + Meropenem	0 (0)	Not observed	20 (40)	Colistin: 1–2 → 1–2 µg/mL; Meropenem: 16–32 → 16–32 µg/mL	30 (60)	Colistin: 0.5–2 → 16–64 µg/mL; Meropenem: 16–32 → 16–32 µg/mL	12	0.002
Colistin + Tigecycline	10 (20)	Colistin: 2–4 → 0.25–0.5 µg/mL; Tigecycline: 1–2 → 0.064–0.125 µg/mL	20 (40)	Colistin: 1–2 → 1–2 µg/mL; Tigecycline: 16–32 → 16–32 µg/mL	20 (40)	Colistin: 0.5–2 → 16–64 µg/mL; Tigecycline: 0.38–1 → 0.38–1 µg/mL		

Association Between Molecular Determinants and Antibiotic Combination Activity in CRAB Isolates

The relationship between carbapenem resistance genes and the effectiveness of antibiotic combination therapy was evaluated among 50 carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates **(**Table 3**)**. Among isolates harbouring the bla_{OXA-51} + bla_{OXA-23} genotype (n = 25), the colistin–tigecycline combination demonstrated synergistic activity in 20% (5/25) of isolates, while 40% (10/25) exhibited indifferent interactions and 40% (10/25) showed antagonistic interactions. In contrast, the colistin–meropenem combination failed to demonstrate synergy in any isolate, with 40% (10/25) showing indifference and 60% (15/25) showing antagonism. Among isolates carrying bla_{OXA-51} + bla_{OXA-23} + bla_{NDM} (n = 10), synergy with the colistin–tigecycline combination was observed in 20% (2/10) of isolates, whereas 50% (5/10) demonstrated indifferent interactions and 30% (3/10) showed antagonistic interactions. Pearson's chi-square analysis demonstrated no significant association between molecular determinants and the interaction outcome ($\chi^2 = 0.833$, df = 4, $p = 0.934$). For the colistin–meropenem combination, no synergistic activity was observed; 50% (5/10) of isolates exhibited indifference, and the remaining 50% (5/10) displayed antagonism. Similarly, among isolates possessing the bla_{OXA-51} + bla_{NDM} genotype (n = 15), the colistin–tigecycline combination exhibited synergy in 20% (3/15) of isolates, indifference in 33.3% (5/15), and antagonism in 46.6% (7/15). No synergistic activity was observed with the colistin–meropenem combination, while 33.3% (5/15) of isolates showed indifferent interactions and 66.6% (10/15) demonstrated antagonistic effects. Owing to the absence of synergy in all groups, Pearson's chi-square assumptions were not met. The Fisher–Freeman–Halton exact test analysis showed **no statistically significant difference** in the distribution of indifference and antagonism among the molecular genotypes ($p > 0.05$). These findings indicate that the presence of bla_{OXA} and bla_{NDM} carbapenemase genes did not significantly influence the occurrence of synergy with colistin–tigecycline, as a consistent 20% synergy rate was observed across all genotypic groups. Furthermore, the absence of synergy and the high frequency of antagonism observed with the colistin–meropenem combination suggest that this regimen may not be effective against CRAB isolates irrespective of their underlying carbapenem resistance genotype.

Table 3
Comparison of drug synergy and molecular determinants of CRAB isolates.

Molecular determinants of CRAB	No. of CRAB isolates	Colistin + Tigecycline ($\chi^2 = 0.833$, df = 4, p = 0.934)			Colistin + Meropenem (p > 0.05)		
		Synergy n (%)	Indifference n (%)	Antagonism n (%)	Synergy n (%)	Indifference n (%)	Antagonism n (%)
bla_{OXA-51} + bla_{OXA-23}	*25*	5 (20)	10 (40)	10 (40)	0	10 (40)	15 (60)
bla_{OXA-51}+bla_{OXA-23}+bla_{NDM}*	10	2 (20)	5 (50)	3 (30)	0	5 (50)	5 (50)
bla_{OXA-51} + NDM	15	3 (20)	5 (33.3)	7 (46.6)	0	5 (33.33)	10 (66.6)

Discussion

The present study highlights the significant burden of *Acinetobacter* spp. infections, with respiratory samples contributing the highest proportion of isolates (32.7%) **(**Fig. 1**)**. This aligns with global evidence showing that *Acinetobacter baumannii* predominantly affects critically ill patients, particularly in respiratory settings such as ICUs [7]. The higher mean age and male predominance found in our study are also in accordance with previous epidemiological findings suggesting increased susceptibility in older and immunocompromised individuals [8].

The present study demonstrates a high prevalence of multidrug resistance among *Acinetobacter spp.*, reflecting the growing global concern regarding this organism as a critical priority pathogen with limited therapeutic options [29, 31]. The antibiotic susceptibility profile revealed substantial resistance to commonly used agents, particularly fluoroquinolones and cephalosporins, with ciprofloxacin (44.2%) and cefepime (43.7%) showing the highest resistance rates **(Fig. 2)**. These findings are consistent with global reports where resistance to these antibiotics frequently exceeds 40–70%, primarily due to mutations in target enzymes, efflux pump overexpression, and β-lactamase production [28, 40]. In the Indian setting, even higher resistance rates to cephalosporins and fluoroquinolones have been reported, often exceeding 60–70% in tertiary care centres, largely attributed to antibiotic pressure and widespread empirical use [35, 34, 9]. Consequently, these antibiotic classes can no longer be considered reliable for empirical therapy in *Acinetobacter* infections. Carbapenem resistance, observed at a rate of 40.2% in the present study, remains a major clinical concern given the role of carbapenems as last-resort antibiotics for multidrug-resistant Gram-negative infections. Global epidemiological data indicate that carbapenem-resistant *Acinetobacter baumannii* (CRAB) is associated with resistance rates ranging from 36–45% in developed regions to over 66–80% in Asia [30, 39, 5]. In India, surveillance studies have shown even higher rates, often exceeding 60–70%, particularly in intensive care units, driven by the widespread presence of carbapenemase enzymes such as OXA-type β-lactamases and metallo-β-lactamases [32, 33]. Although the resistance rate observed in the present study is comparatively lower, it still represents a substantial burden of CRAB. This resistance profile also correlates with findings from the combination therapy analysis, where the colistin–meropenem combination failed to demonstrate synergy, indicating reduced efficacy of carbapenems in resistant isolates. Moderate resistance was also observed for aminoglycosides, including amikacin (40.7%) and gentamicin (36.2%), which is consistent with both national and international resistance trends. Aminoglycoside resistance in *Acinetobacter spp.* is largely mediated by aminoglycoside-modifying enzymes, 16S rRNA methylation, and efflux pump activity [31, 38]. Similarly, resistance to β-lactam/β-lactamase inhibitor combinations such as piperacillin–tazobactam (41.1%) and cefoperazone–sulbactam (35.0%) indicates diminishing efficacy of these agents. Although sulbactam possesses intrinsic activity against *Acinetobacter*, the increasing resistance suggests the involvement of multiple resistance mechanisms, including β-lactamase production and efflux activity. These findings further highlight the limited effectiveness of conventional antimicrobial therapies in multidrug-resistant infections. In contrast, relatively lower resistance rates were observed for minocycline (20.1%) and levofloxacin (27.6%), suggesting partial preservation of activity for these agents. Global studies have similarly reported that tetracycline derivatives such as minocycline and tigecycline retain significant activity against multidrug-resistant *Acinetobacter*, with susceptibility rates often exceeding 80–90% [41, 42, 7, 16]. The retained efficacy of minocycline may be attributed to enhanced intracellular penetration, stability against common resistance mechanisms, and activity against biofilm-forming organisms. However, emerging resistance has been reported, primarily mediated by efflux pumps such as TetB and AdeABC systems, which reduce intracellular drug accumulation [42, 40]. Therefore, although these agents represent promising alternatives, their use should be guided by susceptibility testing to prevent further resistance

development. The overall resistance pattern observed in this study indicates a multidrug-resistant phenotype characterised by concurrent resistance across multiple antibiotic classes, including fluoroquinolones, cephalosporins, carbapenems, and aminoglycosides. This multidrug resistance is driven by the remarkable genetic plasticity of *Acinetobacter spp.*, enabling the acquisition and dissemination of resistance determinants through mobile genetic elements [31]. Indian studies have reported multidrug resistance rates exceeding 70–80%, particularly in ICU settings, contributing to significant clinical challenges [32, 38]. The persistence of such resistant strains in hospital environments contributes to increased treatment failure, prolonged hospitalisation, and higher mortality rates, thereby reinforcing the classification of *Acinetobacter baumannii as* a critical priority pathogen by the World Health Organization [29]. When compared with global and Indian data, the findings of the present study demonstrate strong concordance with established resistance trends. High resistance to fluoroquinolones and cephalosporins aligns with global antimicrobial resistance patterns [28, 30]. while the moderate carbapenem resistance reflects the growing burden of CRAB infections in India [32, 33]. The relatively lower resistance to minocycline observed in this study is also consistent with international evidence suggesting retained activity of tetracycline derivatives [41]. However, the gradual emergence of resistance to these agents emphasises the need for cautious and rational antibiotic use.

The predominance of OXA-type carbapenemases, especially OXA-23, in the current isolates **(Fig. 4d)** is consistent with global molecular epidemiology, where OXA-23 remains the most widespread carbapenemase among CRAB isolates [8]. The co-existence of bla_{OXA-51}, bla_{OXA-23}, and NDM parallels findings from genomic surveillance studies showing increasing detection of strains carrying multiple carbapenemase genes, contributing to both resistance severity and outbreak potential [10, 24, 25]. The detection of NDM among a substantial proportion of isolates is particularly concerning, as NDM-producing strains display high levels of resistance to nearly all conventional β-lactams. Similar patterns of NDM co-occurrence with OXA-type genes have been documented in Asian hospital outbreaks, demonstrating the rapid adaptability and genetic plasticity of *A. baumannii* [10]. The CarbAcineto NP test confirmed carbapenemase activity in 87.5% of phenotypically tested isolates, demonstrating its utility in settings where rapid detection is critical. Previous evaluations of immunochromatographic and rapid phenotypic assays similarly reported high sensitivity and specificity in detecting OXA-23 and NDM enzymes [9], supporting the reliability observed in the present study.

The antagonism observed in 60% of isolates with the colistin + meropenem combination contrasts with earlier clinical optimism regarding potential synergy. Updated clinical evidence shows that carbapenem–polymyxin synergy is inconsistent and often not reproducible across strains, particularly in high-level carbapenemase producers [11]. While some *in vitro* studies have demonstrated reductions in MIC values with colistin–meropenem combinations [43], others have shown no additional clinical benefit compared to colistin monotherapy [45, 50]. Meta-analyses further suggest that the addition of meropenem does not significantly improve mortality or clinical outcomes in multidrug-resistant *Acinetobacter baumannii* infections [45, 50]. The high rate of antagonism observed in the present study may be attributed to pharmacodynamic incompatibility between membrane-disrupting agents such as colistin and cell wall-targeting β-lactams or to adaptive resistance mechanisms induced under antibiotic stress conditions. These findings reinforce the growing evidence that carbapenem-based combinations may be ineffective in highly resistant strains and should not be used empirically without *in vitro* confirmation. The colistin–tigecycline combination demonstrated a heterogeneous response, with 20% of isolates showing synergy, while others exhibited indifference or antagonism **(Fig. 5 and** Table 2**)**. These mixed outcomes are also reflected in multiple global studies showing variable interaction patterns depending on strain-specific resistance determinants, tigecycline MIC levels, and efflux pump expression [7, 43, 44, 46]. As reported in recent antimicrobial stewardship guidelines, tigecycline may be used selectively but is not universally reliable for combination therapy in CRAB due to heterogeneous synergy results [11]. Additionally, resistance mechanisms such as efflux pump overexpression (e.g., the AdeABC system) and ribosomal protection may further reduce tigecycline efficacy, thereby influencing the overall interaction profile. Despite these limitations, combination therapy involving colistin and tigecycline remains a widely explored strategy due to its potential to overcome bacterial resistance and improve antimicrobial activity [48, 49].

The present study also evaluated the association between carbapenem resistance determinants and the efficacy of colistin-based antibiotic combinations among carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates. The findings indicate that molecular determinants, particularly bla_{OXA-23} and bla_{NDM}, did not significantly influence the occurrence of synergistic interaction, especially for the colistin–tigecycline combination. Across all genotype groups, a consistent synergy rate of 20% was observed, suggesting that genotypic variation does not necessarily translate into differences in combination activity **(**Table 3**)**. This observation is consistent with previous reports highlighting that while carbapenemase genes define resistance profiles, they do not reliably predict antibiotic interaction outcomes [31, 32]. A key finding of this study is the absence of synergy for the colistin–meropenem combination across all determinant groups, irrespective of gene profile. The predominance of antagonism, particularly among isolates harbouring

bla$_{OXA-51}$+*bla*$_{OXA-23}$ (60%) and *bla*$_{OXA-51}$+*bla*$_{NDM}$ (66.6%), suggests that carbapenem-based combinations have limited therapeutic value in CRAB infections. These results are supported by global surveillance data demonstrating that OXA-23 is the most prevalent carbapenemase contributing to high-level resistance and treatment failure with carbapenems [1, 40]. Similarly, the presence of NDM, a metallo-β-lactamase, further compromises carbapenem activity, and co-production does not restore antibiotic efficacy [38, 31]. The lack of synergy and high frequency of antagonism observed with colistin–meropenem aligns with findings from earlier studies, which report inconsistent or unfavourable outcomes with carbapenem–polymyxin combinations in CRAB infections [33, 37]. This highlights an important clinical implication that empirical use of carbapenem-containing combinations may not only be ineffective but could potentially result in antagonistic effects, thereby compromising treatment outcomes. Current antimicrobial stewardship guidelines also discourage the routine use of carbapenems in carbapenemase-producing organisms due to their limited efficacy [29].

In contrast, the colistin–tigecycline combination demonstrated a modest but consistent level of synergy (20%) across all molecular determinant groups, suggesting that tigecycline retains partial effectiveness irrespective of carbapenemase gene presence (Table 3). This observation is in accordance with prior studies showing that tetracycline derivatives, including tigecycline and minocycline, exhibit relatively preserved activity against CRAB isolates [41, 42]. However, despite the presence of synergy, the majority of isolates exhibited indifferent or antagonistic interactions, indicating that this combination is not universally effective. The variability in combination outcomes may be explained by the complex resistance mechanisms of *Acinetobacter baumannii*, which extend beyond carbapenemase production. Mechanisms such as efflux pump overexpression, alterations in outer membrane permeability, and biofilm formation significantly influence antibiotic penetration and intracellular activity [40]. In particular, the AdeABC efflux system has been implicated in reduced tigecycline susceptibility, contributing to inconsistent synergy across isolates with similar genotypes [28]. Interestingly, the *bla*$_{OXA-51}$+*bla*$_{NDM}$ group demonstrated a higher proportion of antagonism (46.6%) for colistin–tigecycline compared to other groups, suggesting a potential influence of metallo-β-lactamase-associated resistance on combination behaviour. However, this difference was not statistically significant, indicating that these variations may not have strong clinical relevance. Similar findings have been reported in genotype–phenotype correlation studies, where molecular determinants showed limited association with *in vitro* synergy patterns [34, 36]. The overall findings of this study reinforce the concept that antibiotic combination efficacy in CRAB is multifactorial and cannot be predicted solely based on resistance genes. Although *bla*$_{OXA-23}$ and *bla*$_{NDM}$ are critical markers of carbapenem resistance, they do not dictate the success of the combination therapy. This underscores the need for phenotypic susceptibility testing and *in vitro* synergy analysis to guide treatment decisions, rather than reliance on genotypic data alone.

Conclusion

This study highlights the growing prevalence of carbapenem-resistant *Acinetobacter* species in a tertiary care hospital in North Kerala and demonstrates the widespread presence of carbapenemase genes, particularly *bla*$_{OXA-51}$, *bla*$_{OXA-23}$, and bla$_{NDM}$. The findings indicate that OXA-type carbapenemases are the predominant mechanism of resistance among the isolates. Evaluation of combination drug therapy revealed limited efficacy, with colistin–meropenem showing predominantly antagonistic interactions and colistin–tigecycline demonstrating synergy in only a subset of isolates. Molecular determinants such as *bla*$_{OXA-23}$ and *bla*$_{NDM}$ do not significantly predict combination therapy outcomes, highlighting the need for phenotypic-guided treatment strategies.

Declarations

Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval:

The study was approved by the Institutional Ethics Committee (IEC) at Malabar Institute of Medical Sciences (MIMS), Kozhikode, North Kerala, India, with Study No. EC/112/24.

Consent to participate:

Informed consent was obtained from all individual participants included in the study.

Author Contribution

SA, VV, and Reshmi: Co-Supervision of the study, methodology development, critical revision of the manuscript for important intellectual content, and overall project administration. VK* Study supervision, critical review of the manuscript, interpretation of microbiological and molecular data, and final approval of the version submitted for publication.

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Figures

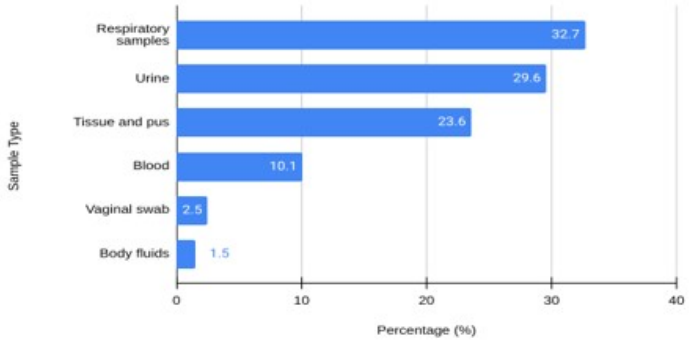


Figure 1

Distribution of *Acinetobacter*isolates by specimen (n = 199).

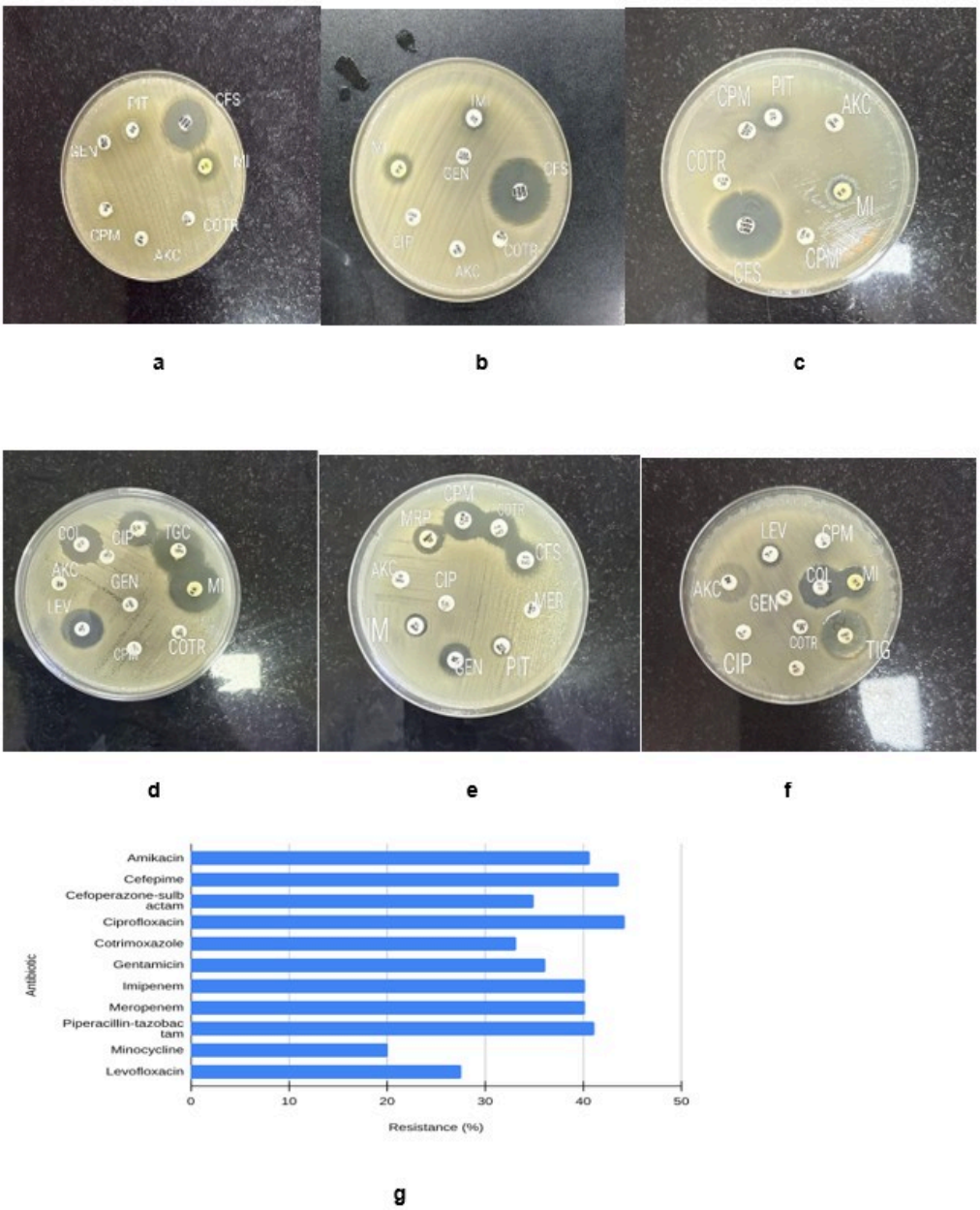


Figure 2

Antimicrobial susceptibility patterns and resistance profile of clinical *Acinetobacter spp.* isolates. a–f. Representative Kirby–Bauer disc diffusion assays performed on Mueller–Hinton agar showing the antimicrobial susceptibility patterns of *Acinetobacter*spp. isolates. **g.** Overall antimicrobial resistance rates of *Acinetobacter*spp. isolates against the selected antimicrobial agents included in the study.

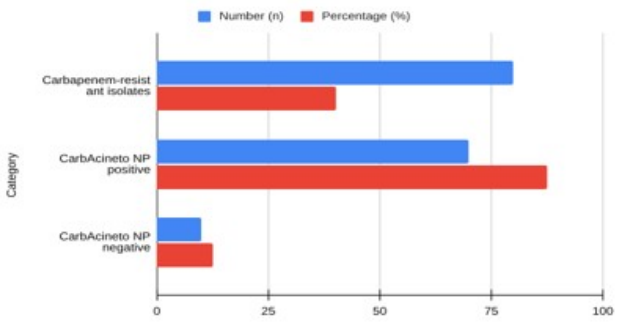


Figure 3

CarbAcineto NP phenotypic confirmation among carbapenem-resistant isolates (n = 80).

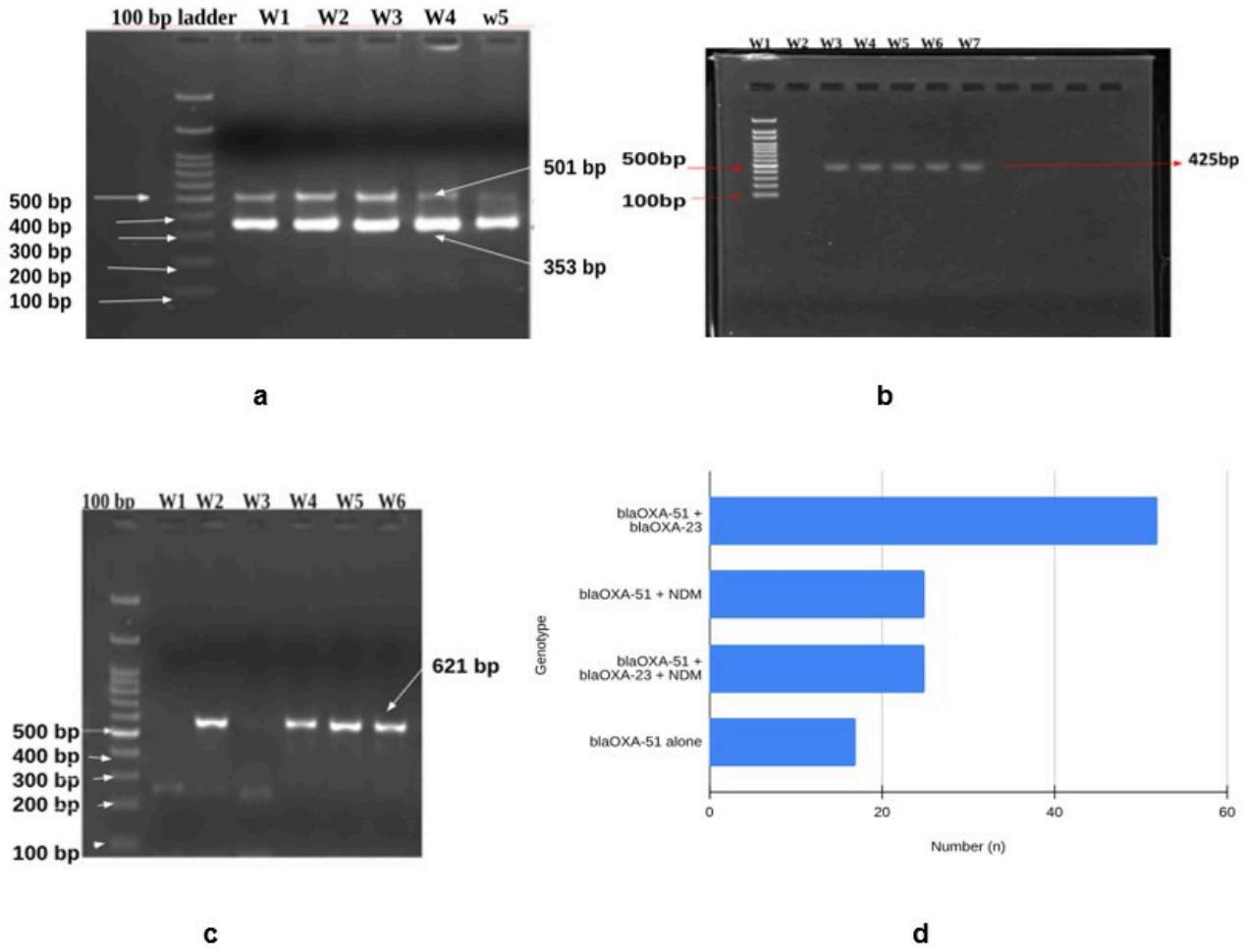


Figure 4

PCR amplification of species-specific (*recA*) and carbapenem resistance genes ($bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}23\text{-}like}$, and bla_{NDM}) in *Acinetobacter* spp. and distribution of carbapenem resistance genes. **a.** Amplification of $bla_{OXA\text{-}51}$ gene (353 bp). Lane 1: 100 bp DNA ladder; Lanes 2–6, 5: Positive samples showing bands at 353 bp and amplification of $bla_{OXA\text{-}23}$ gene (501 bp). Lane 1: 100 bp DNA ladder; Lanes 2–6: 5 positive samples showing bands at 501 bp. **b**. showing amplification of the *recA gene* (425 bp) for confirmation of *Acinetobacter spp.* Lane 1: 100 bp DNA ladder; Lanes 3–7: 5 Positive samples showing distinct bands at 425 bp. **c**. amplification of bla_{NDM} gene (621 bp). Lane 1: 100 bp DNA ladder; Lanes 3, 5–7: 4 Positive samples showing bands at 621 bp. **d** Distribution of carbapenem resistance genes.

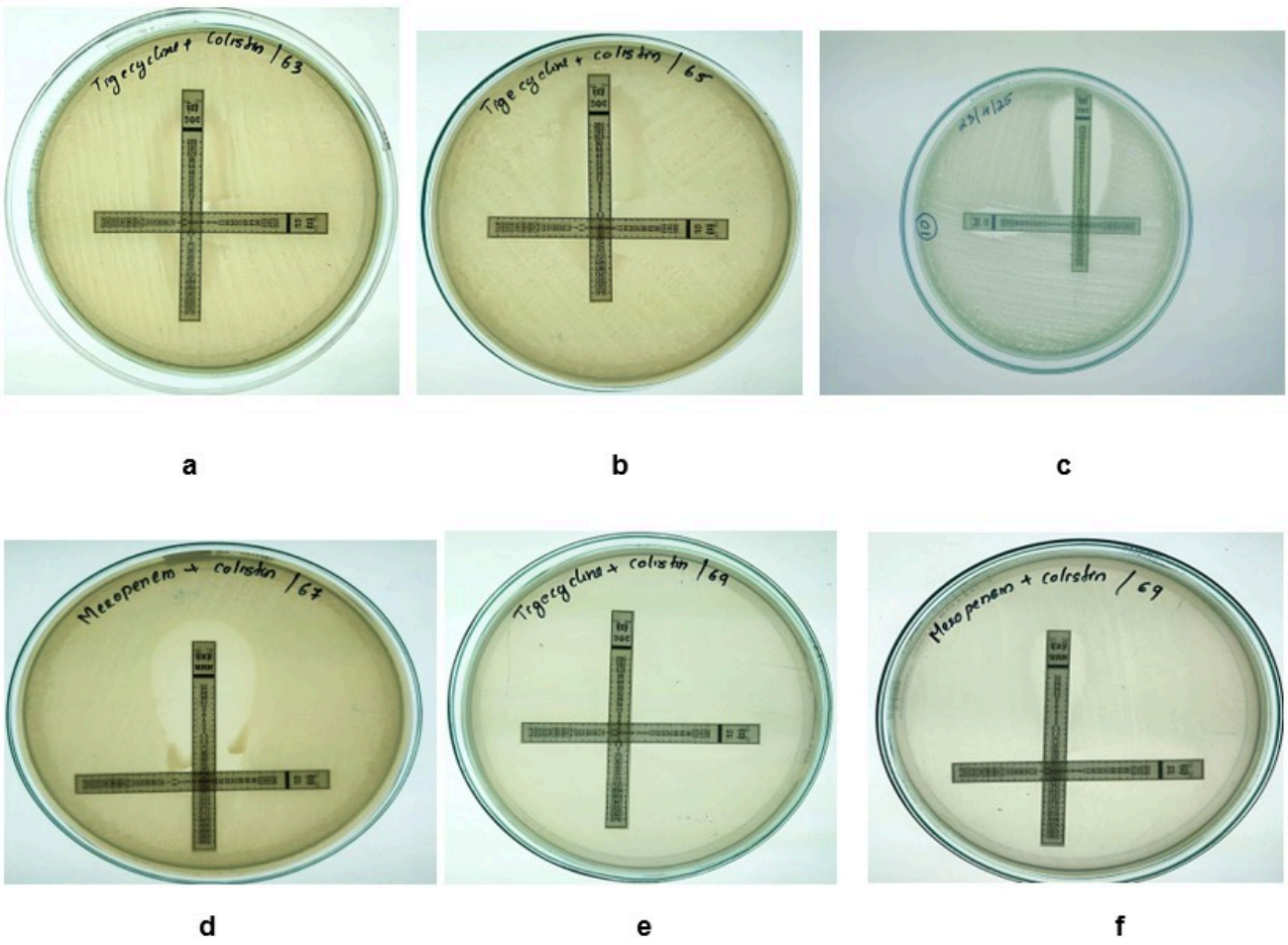


Figure 5

(a–f). Representative E-test (Epsilometer) results illustrating the *in vitro* interactions of colistin–meropenem and colistin–tigecycline combinations against carbapenem-resistant *Acinetobacter*spp. isolates, showing synergistic **(a, b)**, indifferent **(c, f)**, and antagonistic effects **(d, e)**.