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Phenotypic and molecular characterization of multidrug-resistant *Escherichia coli* strains carrying resistance genes isolated from various clinical cases in Iraq

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Abstract

Objective

Multidrug-Resistant (MDR) *E. coli* are among the most important causes of urosepsis. and they also pose a significant economic burden on hospitals worldwide. To further exacerbate the issue, MDR has spread rapidly through *E. coli* populations, making infections more troublesome and costlier to treat, requiring a new therapeutic approach. This investigation aimed to detect the phenotypic and molecular characteristics of MDR in *E. coli* strains recovered from Iraqi patients with urinary tract infections (UTIs) and wounds.

Materials and methods

Thirty clinical samples were collected from midstream urine (MSU) and wound discharge. The clinical E. coli was identified phenotypically based on shape, size, texture, and color of bacteria on medium and confirmed using the VITEC 2 compact system, with 10 confirmed as MDR strains showing 100% resistance to CIP and CAZ. The minimum inhibitory concentration (MIC) testing was done using the checkerboard microdilution method. Fractional Inhibitory Concentration

(FIC) indices were investigated. Gene expression study was preformed via RT-qPCR for *emrD* and *marA* genes. Statistical analysis was achieved using SPSS 27 and GraphPad Prism 10.

Results

The CIP-CAZ combination decreases the MIC level from 200 µg/mL and 260 µg/mL (separately) to 50 µg/mL (combined), with a ΣFIC index of 0.4495, representing highly synergism. Combinations (CIP, CAZ) with bacteriocins and leucine promote greater antibacterial activity, mainly at 50-60 µg/mL, with significant reductions in growth rate ($P < 0.01$). Gene expression revealed noticeable downregulation of *emrD* and *marA* in all combination therapies. For *emrD* gene, the expression decreased from 1.06±0.36 (control) to 0.05±0.05 (CIP/leucine), while for *marA* gene reduced from 1.17±0.47 to 0.03±0.02 (CAZ/bacteriocin), with all comparisons showing P-values < 0.01.

Conclusions

The outcomes support the use of antibiotic-adjuvant combinations as a promising strategy to prevent MDR *E. coli* infections. The result aligns with global studies on antimicrobial synergy and announces novel gene targets for resistance variation.

Keywords: Antibiotic combination, Ceftazidime, Ciprofloxacin, *emrD*, *marA*

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Introduction

A rising percentage of pathogenic isolates in humans show resistance to antibiotics or demonstrate multidrug-resistant ability, generating a significant complication to effective infection cure (Alara and Alara, 2024). Many of these complications have developed due to the rise and extent of multidrug resistant Gram-negative bacteria (MDR-GNB), which have gained resistance against all existing therapeutic types, leading to a modification in handling strategies (Macesic et al., 2025). MDR *Escherichia coli*, an eminent species among MDR-GNBs, is a common threat to healthcare of the global systems. Numerous illnesses that affect people worldwide can be caused by this strain line of MDR *E. coli* and sometimes it can be the major reason of incurable infections especially in burn injuries and sepsis (Zhu et al., 2025). Therefore, developing a successful strategy to treat MDR infections is definitely essential. Combinations between different antibiotics are one way to successfully combat the MDR phenotype. Both CIP and CAZ exhibit broad-spectrum action and resistance to numerous pathogenic bacteria (Elshobary et al., 2025). The CAZ prevents cell-wall synthesis through binding to the PBPs (penicillin binding protein), mostly PBP3 in Gram-negative bacteria. Similarly for CIP, it blocks the enzymes DNA gyrase and topoisomerase IV, which are necessary for the replication DNA in bacteria (Dabhi et al., 2024). MDR bacteria can develop resistance to both CAZ and CIP, which increase a major challenge in evolving functional cures to prevent the spread of MDR bacteria (Wang et al., 2024). This resistance may be brought on through the improvement of the beta-lactamase enzyme, which destroys ceftazidime's beta-lactam ring and inhibits it from inducing the building of cell walls (Obaid et al., 2025). Furthermore, CIP and other drugs may not be active against MDR bacteria if genetic abnormalities in the genes encoding the gyrase and topoisomerase IV enzymes prevent them from attaching to their targets (Collins, and Osheroff, 2024). Developing the number of efflux proteins, which transfer antibiotic act outside the cell membrane, is alternative way that several bacteria form resistance. Combination of two antibiotics has been proved in previous research to significantly decrease the growing of MDR bacteria that are resistant to many antibiotics (Gonçalves et al., 2025). CIP and CAZ are two such combinations that can apply for some serious infections. Nevertheless, the kind of bacteria strain and their resistance pattern determine this interaction. Furthermore, some studies have indicated that this combination may be ineffective in some instances and for specific infections (Ngo et al., 2024). Therefore, other compounds produced from non-pathogenic isolates have been tested, such as bacteriocins, which have proven highly effective against pathogenic bacteria, especially when combined with antibiotics (Chen et al., 2024). Furthermore, the effectiveness of some antibiotics can be increased when combined with non-antibacterial compounds, such as amino acids, which may facilitate or deceive bacteria into reducing resistance and thus eliminating those (Han et al., 2021). Our current research aims to study the effect of the CAZ-CIP, CAZ-bacteriocin, CAZ-leucine, CIP-bacteriocin, and CIP-leucine combinations on gene expression, which is advantageous for its medical use. In this study, we carried out extensive surveys using an MDR *E. coli* bacterium. Through in vitro testing for antimicrobial susceptibility, we evaluated the minimum inhibitory concentration (MIC) for the following combinations: CAZ-CIP, CAZ-bacteriocin, CAZ-leucine, CIP-bacteriocin, and CIP-leucine. To conclude the impacts of the mentioned combinations, we extracted the mRNA, measured its purity and concentration, and then transformed it into cDNA. Gene expression was subsequently measured using RT-qPCR.

Materials and methods

Study setting: The investigations were conducted by the microbiology laboratory at the Department of Medical Biotechnology at Al-Qasim Green University, located in the South-Central region of Babylon province, Iraq. According to standardized clinical procedures, clinical samples were collected aseptically at medical hospitals throughout Babylon province, Iraq. All information was removed from the specimens before processing in the laboratory to ensure confidentiality of the data.

Study design: In this cross-sectional study design. Between January and December of 2025, we collected 30 samples from patients with urinary tract infections and others suffering from wound discharge. Samples were collected by nurses working in hospitals in Babylon province and processed at the Department of Medical Biotechnology, College of Biotechnology, Al-Qasim Green University, Babylon, Iraq. The identification workflow integrated conventional bacterial culture, molecular detection through the RT-qPCR panel, and assessment of minimum inhibitory concentrations for each combination. These studies were conducted according to the guidelines of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) for observational studies.

Specimen collection: In this study, random specimens were collected to obtain a representative specimen. Midstream urine (MSU) samples were collected from all female patients using sterile, dry, and leak-proof 20 mL containers. As for wound discharge samples, they were collected from the female patients using Amies Transport Media. All the patients were females, aged between 18 and 50 years. The samples were collected from the urinary department at Yarmouk Hospital, Baghdad, Iraq. Afterwards, labels containing patient information about the specimen were attached, and specimens were quickly transported to the laboratory, analyzed, and cultured within 2h after collection. To prevent contamination. Various qualitative and quantitative methods were followed in this study.

Ethical considerations: The study was carried out in accordance with the guidelines outlined in the Declaration of Helsinki, and the subject information and the consent form were reviewed and approved by the Al-Qasim Green University Ethical Committee according to document number M311 on January 23, 2025.

Bacterial procedures: For bacterial identification, Eosin Methylene Blue and MacConkey agar were used as selective culture media then the isolates were transferred to nutrient broth for activation and identification using the Vitek2 compact system. After identifying the bacteria, antibiotic sensitivity testing was performed on all isolates to determine their resistance to multiple antibiotics (Ojaimi and Shliouh, 2025).

Production and purification of bacteriocin: The procedure for identifying the isolate that can produce bacteriocin, along with the comprehensive methods for its production, extraction, purification, and assessment of its efficacy against pathogenic bacteria, was completed and documented in the earlier research paper.

Assessment of MIC for each combination: After performing antimicrobial susceptibility testing for six different antibiotics, such as Ceftazidime (CAZ), Aztreonam (ATM), Imipenem (IPM), Gentamicin (CN), Azithromycin (AZM), and Ciprofloxacin (CIP) against 30 samples of *E. coli*, 10 samples were found to be resistant to ≥3 antimicrobial classes, called MDR bacteria. Remarkably, all bacterial isolates showed 100% resistance to CIP and CAZ across the six antibiotics tested. To determine the MICs, these two antibiotics were combined at different concentrations (20, 30, 40, 50, 60, 70, 80, and 90 µg/mL). The MIC testing of the combined drugs was conducted using microtiter plates, which were incubated overnight at 37°C. After incubation, the results were measured at 600 nm using an ELISA reader. Subsequently, CIP and CAZ were evaluated at concentrations of 30, 40, 50, and 60 µg/mL when combined with bacteriocin at 125 µg/mL, this concentration was previously identified as MICs in our previous research (Al-Bderee et al., 2025), and with leucine amino acid at 10 mM. The test was also done on microtiter plates and the results were read at 600 nm using ELISA reader. MIC was determined accordingly the instructions described in the (CLSI, 2022).

Assessment of the fractional inhibitory concentration (FIC) for each combination of antibiotics: The combination effect of CIP and CAZ was evaluated via checkerboard microdilution procedure (Jassim et al., 2024). The MIC for each CIP and CAZ were individually identified using broth microdilution in 96-well plates. Both antibiotics were mixed in a checkerboard preparation with serial dilutions. Each well contained a different concentration pairing of the antibiotics in addition to being inoculated with a same bacterial suspension. The plates were incubated at 37°C for duration of 18 to 24 hours.

FIC calculation: FIC_CIP = MIC_CIP in combination / MIC_CIP alone, and FIC_CAZ = MIC_CAZ in combination / MIC_CAZ alone.

Interpretation: A ΣFIC ≤ 0.5 indicates synergism, confirming enhanced activity when CIP and CAZ are used together.

Study of gene expression in *E. coli* for each combination: The expression levels of the *emrD* and *marA* genes were assessed following each combination treatment. The expression analysis was done using RT-qPCR (Moradi et al., 2025). Briefly, total RNA was extracted from all isolate using the RNeasy Mini Kit (Qiagen), adhering closely to the manufacturer's specified guidelines. To remove the DNA from the extracted RNA, DNase from Fermentas, USA was used. The purity of the RNA was evaluated by formaldehyde-denaturing 1.2% (w/v) agarose gel-

electrophoresis. RNA concentrations and absorbance ratios at A260/A280 and A260/A230 were determined using the Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies Inc.). Following the manufacturer's recommendations, 0.5 μg of total RNA was converted into single-stranded cDNA using Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase and random hexamer oligonucleotides (Fermentas). The *emrD* and *marA* genes were then amplified from the created cDNA. The primer sequences are listed in Table 1. Real-time PCR was conducted using a Bio-Rad MiniOpticon™ device with the Fermentas SYBR Green qPCR Master Mix. The thermocycler program and reaction conditions used in this research involved an initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 10 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes for each primer. The comparative Ct method (2^-ΔCt formula) was employed to calculate the expression levels of the *emrD* and *marA* genes after normalizing with the *GADPH* gene (Jawad et al., 2024).
ΔCt = Ct (gene of interest) - Ct (housekeeping gene).
ΔΔCt = ΔCt (treated sample) - ΔCt (untreated sample).
Fold gene expression = 2^-(ΔΔCt)

Table 1. The study employed primers, including their oligonucleotide sequences and the sizes of the amplicons

Gene names	Sequences (5'-3')	product Size (bp)
emrD	F: GCTGCTGATGTTGCTGTTGA R: CGATGACGATGCGTTCTTCT	140bp
marA	F: CGTCTGCTGTTGCTGATGTT R: GCTGATGCGTTCTTCTTGGT	150bp
GAPDH (Housekeeping gene)	F: ACTTACGAGCAGATCAAAGC R: AGTTTCACGAAGTTGTCGTT	170bp

Statistical analysis: Statistical calculation and graphical representation were conducted using SPSS 27 and GraphPad Prism 10, employing a t-test to conclude significance between the two groups. In contrast, ANOVA is used to check significance among three or more groups. A P-value is considered significant if $P < 0.05$.

Results and discussion

***E. coli* isolation and confirmation:** All samples revealed were *E. coli*, and no other bacteria were found. For more accuracy, the bacterial isolates were confirmed via using the Vitek2 compact system. The results were consistent with culture identification results, confirming that they were *E. coli* with a probability of 99%.

The combined impact of CIP and CAZ: The MIC of CIP and CAZ were measured individually and in combination utilizing the checkerboard microdilution method. The MIC for CIP and CAZ were 200 µg/mL, and 260 µg/mL respectively. In combination, both antibiotics showed reduction in MIC of 50 µg/mL each. The FIC values for CIP and CAZ were 0.2462 and 0.2033 respectively, resulting in a total FIC index of 0.4495. Based on interpretive guidelines, this index conforms a high synergistic effect between the two antibiotics against the tested bacterial strain.

Antibacterial effect investigation for all combinations: As mentioned previously 10 isolates diagnosed as MDR were evaluated to study the effects of different concentrations of CIP and CAZ combination against *E. coli*. After applying triple independent tests for the same sample, the MIC for the combination was establish to be 50 µg/mL, as shown in Table 2. Bacteriocin concentration was predetermined at 125 µg/mL before use in combination. The MIC for the CIP and CAZ combination showed a decrease in bacterial growth, directly related to the dose used. It started at 0.191±0.049 at 20 µg/mL and gradually decreased to 0.100±0.041 at 90 µg/mL. The control group showed a higher MIC of 0.491±0.91, and the overall p-value of less than 0.01 indicates the statistical significance of the observed changes. These findings presented that the combinatorial therapy is more effective than mono-antibiotic therapy, indicating a synergistic effect between the two antibiotics. In Table 2, the study of MIC followed by post-hoc testing specified that a high significant differences among treatment groups, p-value < 0.01. The alphabetical labeling to indicate the statistical similarities or differences highlights the accuracy of the comparisons: groups having same letter are statistically similar, while others with differing letters show significant variation in antimicrobial efficiency. This result assumes the probability that CIP and CAZ vary in their inhibitory competences, potentially because of the various ways of action or resistance properties in the *E. coli* strain.

Table 2. Results of CIP (Ciprofloxacin)/CAZ (Ceftazidime) OD determination/ the analysis of OD followed by post-hoc testing

Concentration	OD (Mean±SD)
	CIP/CAZ
20.00	0.191 ± 0.049^{B}
30.00	0.17 ± 0.04^{BC}
40.00	0.146 ± 0.04^{BD}
50.00	0.110 ± 0.05^{CD}
60.00	0.102 ± 0.04^{CD}
70.00	0.101 ± 0.05^{CD}
80.00	0.100 ± 0.04^{CD}
90.00	0.100 ± 0.041^{D}
Control	0.491 ± 0.91^{A}
P-value	<0.01

Furthermore, investigation effects of bacteriocin (125 μg/mL) and leucine (10 mM) combining with either CAZ or CIP at several concentrations (30, 40, 50, and 60 μg/mL) showed an interested outcome. The results noticeably indicate a reduction in bacterial growth depending on combination dose. These effects provide substantial support to the theory that bacteriocins increase the antibiotics effectiveness, especially at higher concentrations (Table 3). This obtained effectiveness may be due to the difference in mechanisms or modes of action between bacteriocin and antibiotics, as bacteriocin affects the cell membrane of the cell and the antibiotic affects the internal systems and vital activities of pathogenic bacteria, which leads to a significant reduction in the growth of these bacteria.

Table 3. Statistical Analysis of Antimicrobial Combinations across Varying Concentrations

Combination	30 μg/mL	40 μg/mL	50 μg/mL	60 μg/mL	P value
1A only bacteria	0.575^{a}	0.571^{a}	0.569^{a}	0.567^{a}	**0.98 NS**
1B bacteria + CAZ	0.513^{a}	0.511^{a}	0.509^{a}	0.507^{a}	**0.97 NS**
1C bacteria + CIP	0.495^{a}	0.494^{a}	0.491^{a}	0.489^{a}	**0.96 NS**
CIP+ bacteriocin	0.237^{bc}	0.194^{c}	0.138^{bc}	0.137^{bc}	**0.003 S**
CAZ +bacteriocin	0.185^{c}	0.167^{c}	0.157^{bc}	0.155^{bc}	**0.015 S**
CIP+leucine	0.281^{b}	0.16^{c}	0.056^{c}	0.057^{c}	**0.01 S**
CAZ+leucine	0.243^{b}	0.24^{b}	0.226^{b}	0.231^{b}	**0.72 NS**
P value	0.0004	0.0003	0.01	0.01	

NS: non-significant, S: significant, for Y axis: same letters indicate no significant while the different letters indicate significantly reduce in bacterial growth. P value ≤ 0.05.

Regarding the combination of leucine with the antibiotic ciprofloxacin, a significant decrease in the growth of pathogenic bacteria was observed, especially with the use of high concentrations of the antibiotic (50 and 60 μg/mL), indicating very high efficacy of this combination. However, with CAZ and leucine combinations, no change in the level of pathogenic bacterial growth was observed, suggesting lower or no efficacy compared to the leucine-CIP combination.

Expression of *emrD* and *marA* genes in *E. coli* in response to various combinations: The fold change in gene expression was determined for all bacterial isolates utilizing the (2^-ΔCt formula). The gene expression level of the *emrD* gene was estimated for the different therapeutic formulations. The untreated control group (1A) and the single-antibiotic-treated isolates (1B and 1C) showed relatively high gene expression levels (where ΔΔCt values were between 1.06±0.36 for group 1A and 0.91±0.45 and 1.43±0.68 for groups 1B and 1C, respectively), indicating slight inhibition under the influence of these treatments. Conversely, combination therapies—CIP/CAZ (0.08±0.11), CIP/leu (0.05±0.05), CAZ/leu (0.13±0.15), CIP/bacteriocin (0.08±0.12), and CAZ/bacteriocin (0.92±0.13)—led to significant down-regulation of *emrD*, with p-values consistently below 0.05. These results indicate that treatment with combined therapeutic compounds disrupts the replication of genetic material by affecting *emrD* units, which directly contributes to inhibiting and reducing bacterial growth, as shown in Table 4.

Table 4. Comparison of Treatments with controls for *emrD* gene

Treatment	$2^{-(\Delta\Delta Ct)}$ Mean±SD	P-value*	P-value**	P-value***
1A	1.06±0.36			
1B	0.91±0.45	0.543		
1C	1.43±0.68	0.085	0.228	
2- CIP/CAZ	0.08±0.11	0.012	0.008	0.002
3- CIP /leu	0.05±0.05	0.002	0.002	<0.01
4- CAZ /leu	0.13±0.15	0.041	0.023	0.004
5- CIP/bacteriocin	0.08±0.12	0.011	0.008	0.002
6- CAZ/bacteriocin	0.92±0.13	0.018	0.012	0.003

* Comparison of each treatment with 1A; ** Comparison of each treatment with 1B; *** Comparison of each treatment with 1C;*** indicate P-value <0.05; **** indicate P-value <0.01.

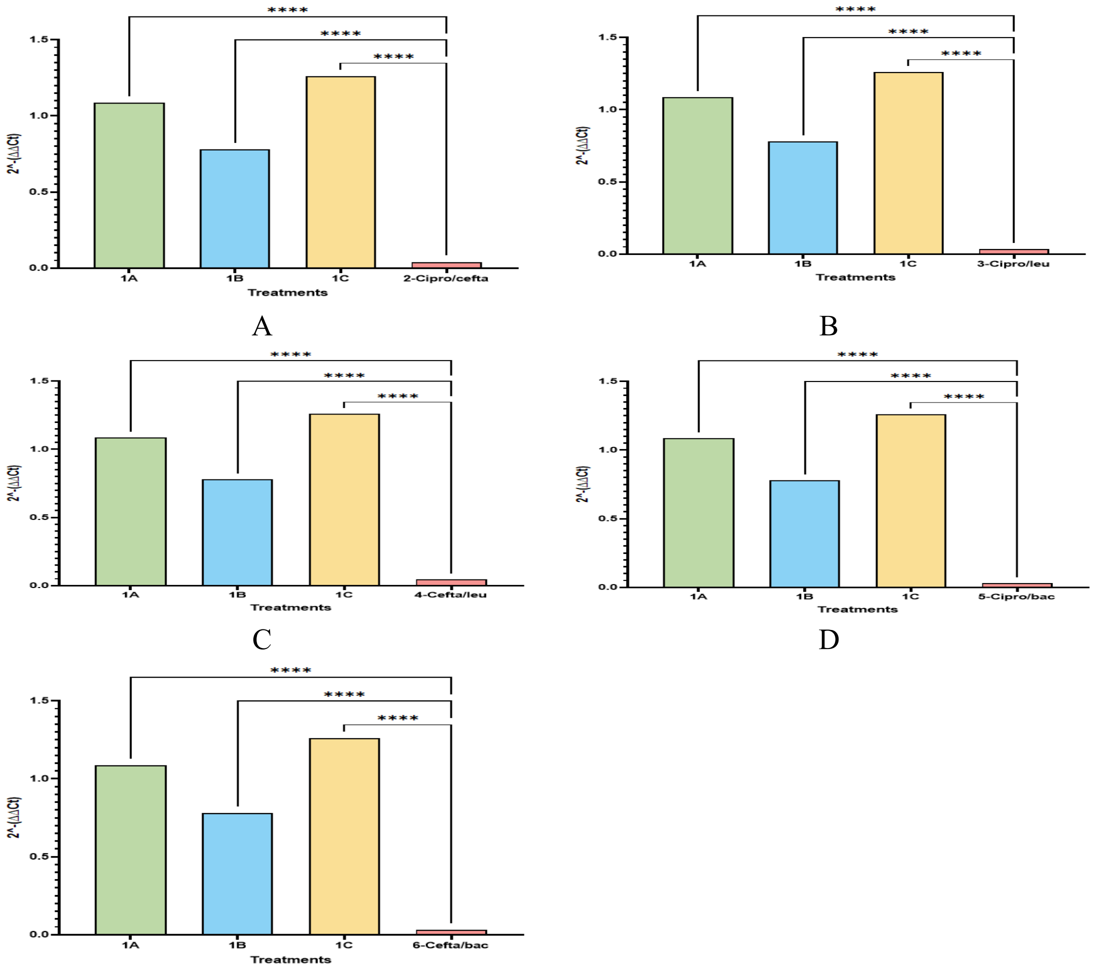
Similarly, the gene expression of the *marA* gene was found to be in the same context. The control and single treatments recorded higher levels (1.17±0.47 for 1A to 1.2±0.9, 1.1±0.74 for 1B and 1C respectively), whereas combination therapies markedly decreased expression: (0.09±0.06) for CIP/CAZ, CIP/leu (0.11±0.08), CAZ/leu (0.06±0.1), CIP/bacteriocin (0.06±0.07), and CAZ/bacteriocin (0.03±0.02). All comparisons showed highly significant p-values (<0.01), reflecting high inhibition of gene expression. Since the *marA* gene plays a crucial role in multidrug resistance, its inhibition reflects the effectiveness of therapeutic combination against pathogenic bacteria, as shown in Table 5.

Table 5. Comparison of Treatments with controls for *marA* gene

Treatment	$2^{-(\Delta\Delta Ct)}$ Mean±SD	P-value*	P-value**	P-value***
1A	1.17±0.47			
1B	1.2±0.9	0.154		
1C	1.1±0.74	0.225	0.693	
2- CIP/CAZ	0.09±0.06	0.006	0.004	0.002
3- CIP /leu	0.11±0.08	0.009	0.005	0.003
4- CAZ /leu	0.06±0.1	0.010	0.005	0.003
5- CIP/bacteriocin	0.06±0.07	0.008	0.005	0.002
6- CAZ/bacteriocin	0.03±0.02	0.003	0.003	0.01

* Comparison of each treatment with 1A; ** Comparison of each treatment with 1B; *** Comparison of each treatment with 1C;*** indicate P-value <0.05; **** indicate P-value <0.01.

Figures 1 and 2 illustrate the molecular effects of these combinations by assessing the gene expression of *emrD* and *marA* using the 2^-(ΔΆCt) method. Control groups consisted of untreated bacteria and bacteria treated once with CIP and once with CAZ, in addition to treatment groups consisting of a combination of these antibiotics and bacteriocins. The results showed a significant decrease in the gene expression levels of both genes in the combinations compared to the single treatments, and these decreases are represented in the figure by the symbols *** and **** for statistical significance. The therapeutic combination significantly reduced the gene expression levels of both *emrD* and *marA*. This suggests they are important in inhibiting important resistance mechanisms, especially those related to efflux activities and gene regulation. The reduction in the gene expression of *emrD*, which helps transport multiple drugs out of the cell, increases the period antibiotics remain inside the cell. This allows the antibiotic to work effectively. Meanwhile, the inhibition of *marA* gene expression, which is key in managing resistance genes, shows that the cell's defense mechanisms fail to adapt well against these combinations. Overall, these formulations effectively limited the growth of *E. coli* bacteria compared to untreated isolates.



A

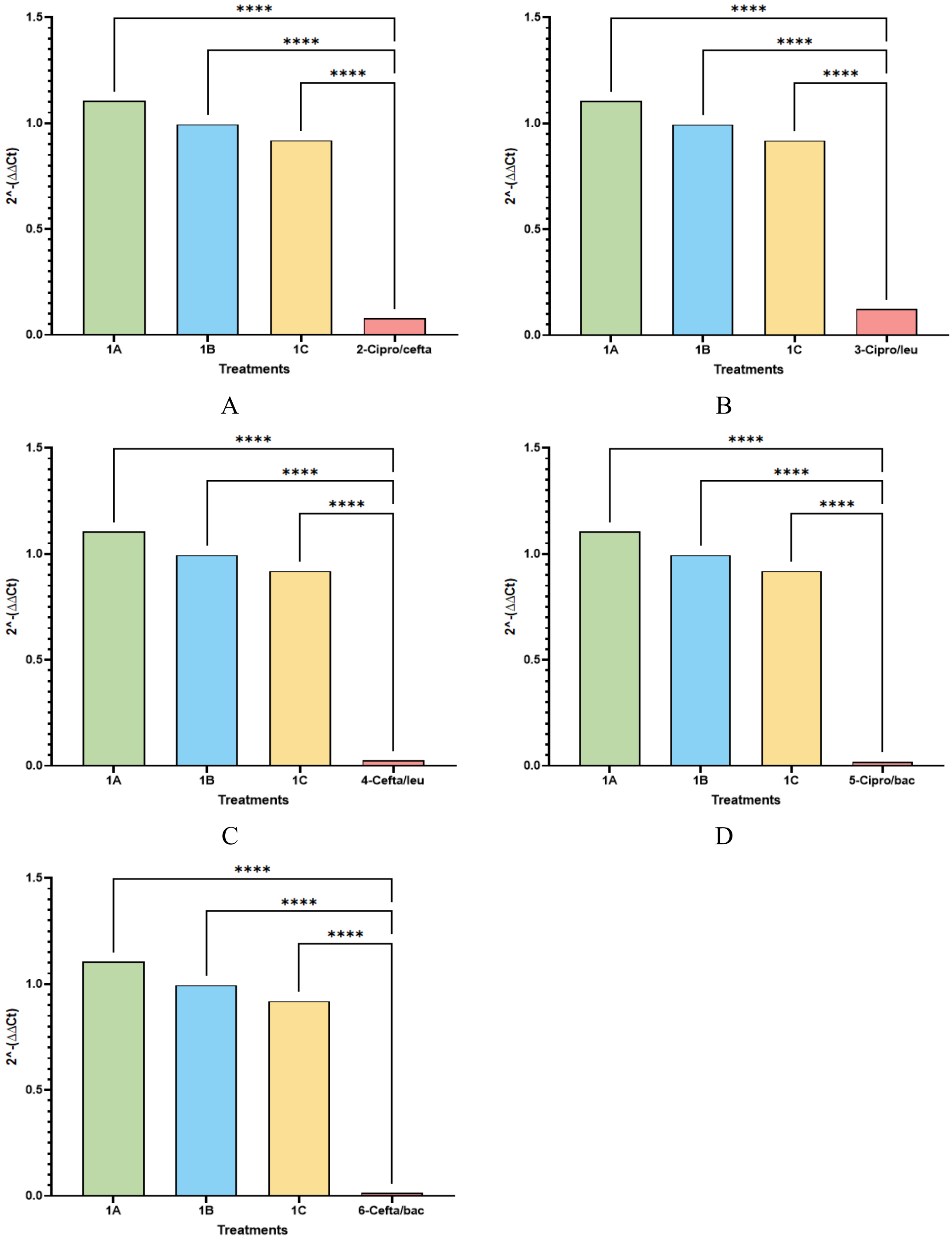
B

C

D

E

Figure 1. Multiple comparison of mean $2^{-(\Delta\Delta Ct)}$ for *emrD* between control groups (1A: only bacteria; 1B: bacteria + CAZ; 1C: bacteria + CIP) with Treatments with Treatments. Multiple comparisons between Cipro/cefta (A) Cipro/leu (B) Cefta/leu (C) Cipro/bac (D) Cefta/bac (E). *** indicate P-value <0.05; **** indicate P-value <0.01



A

B

C

D

E

Figure 2. Multiple comparison of mean 2-(ΔΔCt) for *marA* between control groups (1A: only bacteria; 1B: bacteria + CAZ; 1C: bacteria + CIP) with Treatments. Multiple comparisons between Cipro/cefta (A), Cipro/leu (B), Cefta/leu (C), Cipro/bac (D), Cefta/bac (E). * indicate P-value <0.05; **** indicate P-value <0.01.**

Comparison between treatments results: The statistical comparison showed that treatments 1A, 1B, and 1C led to higher expression levels of both *emrD* and *marA* genes (Table 6). There were no significant differences among these treatments. In contrast, combinations like CIP with CAZ, leucine, or bacitracin (groups 2-6) significantly reduced gene expression levels. The P-values (<0.01) confirm that there are significant differences between the control and treatment groups (Figures 3 and 4).

Table 6: Statistical Comparison of Treatments for Each Gene

	1A	**1B**	**1C**	**2-Cipro/cefta**	**3-Cipro/leu**	**4-Cefta/leu**	**5-Cipro/bac**	**6-Cefta/bac**	**P-Value**
emrD	1.06±0.36^{ab}	0.91±0.45^{b}	1.43±0.68^{a}	0.08±0.11^{c}	0.05±0.05^{c}	0.13±0.15^{c}	0.08±0.12^{c}	0.92±0.13^{c}	<0.01
marA	1.17±0.47^{a}	1.2±0.9^{a}	1.1±0.74^{a}	0.09±0.06^{b}	0.11±0.08^{b}	0.06±0.1^{b}	0.06±0.07^{b}	0.03±0.02^{b}	<0.01

Different letters indicate statistically significant differences between treatments, Similar letters indicate statistically non-significant differences between treatments

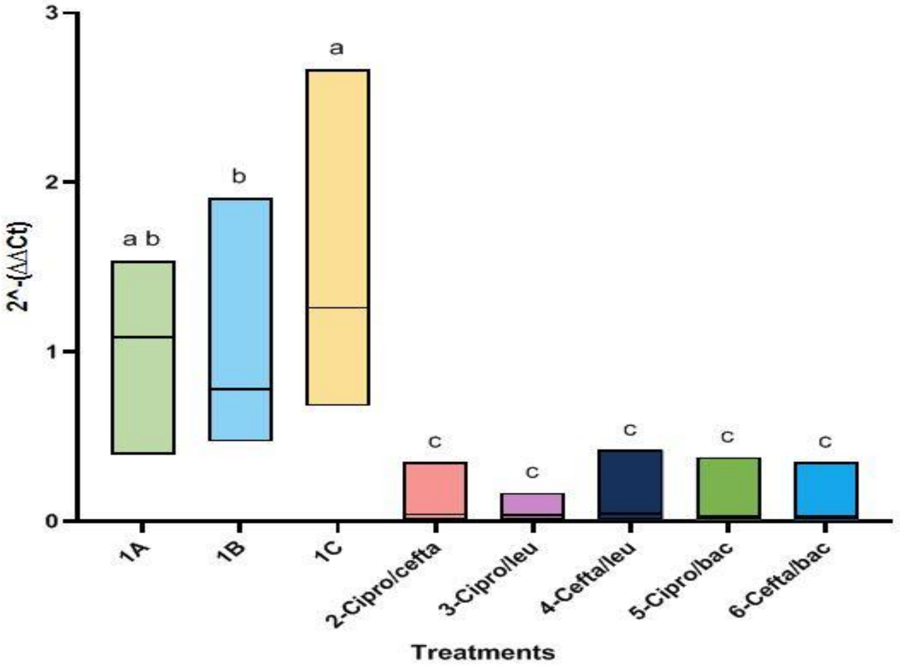


Figure 3. Visual representation of $2^{-(\Delta\Delta Ct)}$ for treatments in *emrD*. Different letters indicate statistically significant differences between treatments; similar letters indicate statistically non-significant differences between treatments

The comparison of gene expression across treatments revealed that the control groups (1A, 1B, 1C) showed no significant differences in *emrD* and *marA* expression levels ($P > 0.5$) (Figure 5). However, combination treatments like Cipro/cefta, Cefta/leu, and Cefta/bac showed significantly different expression levels between the two genes, with P-values < 0.05. These findings suggest that there are gene-specific responses to antimicrobial combinations.

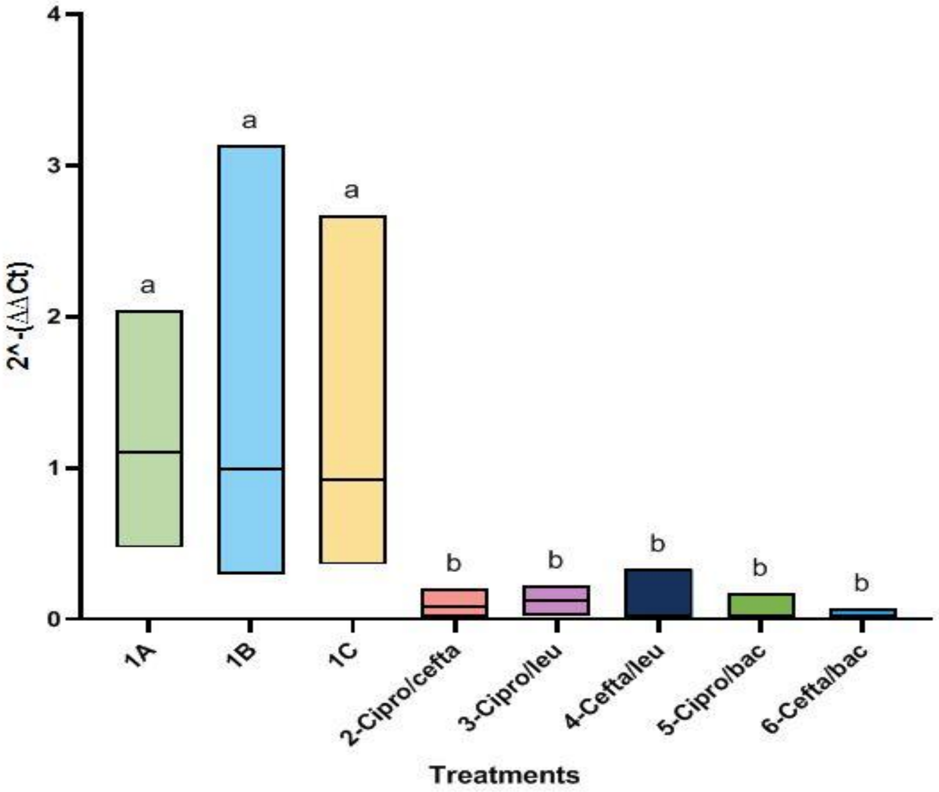


Figure 4. Visual representation of $2^{-(\Delta\Delta Ct)}$ for treatments in *marA*. Different letters indicate statistically significant differences between treatments; similar letters indicate statistically non-significant differences between treatments

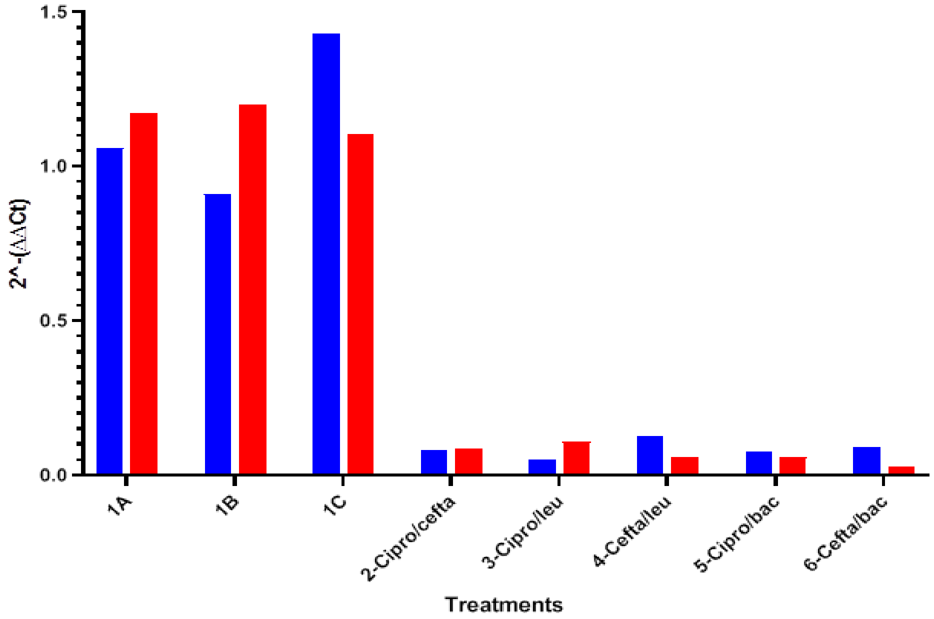


Figure 5. Visual representation of 2^-(ΔΔCt) of Genes for Each Treatment (red color for *marA* gene and the blue for *emrD)*

Comparison between isolates: The statistical comparison of *ermD* and *marA* gene expression across ten bacterial isolates revealed no significant differences between the two genes (Table 7). Although the mean expression values varied slightly across isolates, the P-values (0.963 for *ermD* and 0.718 for *marA*) indicate that these differences are not statistically significant. This suggests that both genes exhibit comparable expression profiles under the tested conditions.

Table 7. Statistical comparison of isolates for each gene

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**P-Value**
emrD	0.38±0.34	0.49±0.59	0.41±0.58	0.46±0.56	0.67±0.85	0.44±0,2137.68	0.39±0.49	0.46±0.57	0.45±0.64	0.34±0.5	0.963
marA	0.33±0.44	0.17±0.19	0.38±0.43	0.56±0.75	0.73±1	0.86±1.2	0.45±0.52	0.54±0.81	0.44±0.59	0.31±0.36	0.718

The present investigation provides exciting evidence that combination treatments involving CIP and CAZ, either alone or in combination with bacteriocins or leucine, significantly prevent the growth of MDR *E. coli*. This is confirmed by the significant drop in MIC values and the statistically significant down-regulation of *emrD* and *marA* gene expression, both of which are related with efflux activity and transcriptional regulation. The inhibition of *emrD*, a multidrug efflux transporter, could increase intracellular antibiotic detention, while the repression of *marA*, an inclusive regulator of resistance genes, recommends a wide breakdown of adaptive resistance pathways. These results match recent global research. For example, Obaid et al. (2025) found that the effectiveness of antibiotics increases when they are used with biological agents that play a key role in cellular metabolism. These agents help antibiotics enter the cell and affect the genes that cause resistance. This lowers their gene expression. Gonçalves et al. (2025) found that antibiotic effectiveness increases when combined with antimicrobial peptides (AMPs), showing strong synergy against MDR bacteria. In this study, the use of bacteriocin, a type of AMP, produced similar results, especially in reducing the growth of MDR bacteria and inhibiting the expression of pathogenic genes. Ngo et al. (2024) confirmed the synergistic effect of the CIP-CAZ combination against *Pseudomonas aeruginosa* with FIC analysis. This study also included *E. coli* to support the broader use of these combinations and the potential to target a wider range of pathogeneic bacteria. Moreover, Chen et al. (2024) confirmed that bacteriocins help maintain membrane integrity and improve antibiotic uptake. This may significantly lower the expression of *emrD*, whose gene expression was assessed in this study. Unlike many earlier studies that focused on genes like *blaTEM*, *GyrA*, and *ompC* (Al-Bderee et al., 2024), this study directly targeted the *emrD* and *marA* genes. This method offers new insights into efflux regulation and gene regulation in MDR *E. coli*. Adding leucine as a non-antibiotic synergistic molecule also brings new contributions (Gálvez-Benítez et al., 2023). Our data show that leucine increases CIP activity, but its effect is limited when paired with CAZ. This suggests a compound-specific interaction, allowing for a better understanding of the mechanisms involved. Additionally, previous studies observed that endolysin and antibiotics can work well together (Xiao et al., 2023). For example, combining colistin with LysABP-01 endolysin, which is produced as an *A. baumannii* phage, resulted in significant growth suppression and a synergistic result (Hong et al., 2022). Other studies have also shown that epigallocatechin gallate (EGCG) inhibits key bacterial enzymes, such as DNA gyrase and dihydrofolate reductase, which are crucial for DNA replication and cell survival (Thummeepak et al., 2016). Some studies also showed that the combination of ceftazidime-avibactam (CAZ-AVI) is one of the first options for the empirical treatment of nosocomial infection with possible involvement of gram-negative bacilli, especially if it presents with severity criteria or occurs in the "fragile" patient. The use of CAZ-AVI also reduces the consumption of Carbapenem (Candel et al., 2022).

Conclusions: The findings of this study highlight the enhanced antibacterial activity of combining traditional antibiotics with bacteriocins and leucine against MDR *E. coli*. Specifically, the combinations of CAZ with CIP, as well as each antibiotic paired with leucine or bacteriocins, significantly inhibited bacterial growth and exhibited synergistic effects ($p \leq 0.05$). These results suggest that such combinations may offer a promising strategy to combat antibiotic resistance. Additionally, targeting bacterial *emrD* and *marA*, the molecular findings suggest that such therapies effectively disrupt efflux mechanisms and transcriptional regulation, thereby enhancing antibiotic retention and weakening bacterial defense systems. These results not only align with global research trends but also introduce novel gene targets and combination strategies that may inform future antimicrobial development. Future research should focus on optimizing these combinations and exploring their potential in clinical applications.

Author Contributions

Raid Razzaq Ojaimi and Nadhim Mushtaq Hashim. performed data analysis, Nawar Al-Janabi. edited the article, Maha Diekan Abbas. oversaw the work, and M.D.A. carried out the experiments. After reading the published version of the article, all writers have given their approval.

Data availability statement

Data are available upon reasonable request.

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Ethical Considerations

The authors avoided data fabrication, plagiarism, falsification, and misconduct.

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Conflicts of interest

There are no conflicts of interest.

References

Alara, J. A., & Alara, O. R. (2024). An overview of the global alarming increase of multiple drug resistant: A major challenge in clinical diagnosis. *Infectious Disorders - Drug Targets*, *24*(3), Article e250723219043. https://doi.org/10.2174/1871526523666230725103902

Al-Bderee, N. M. H., Al-Janabi, N., & Salih, H. S. (2025). The molecular effects of biosynthesized colicin on pathogenic bacteria. *Journal of Microbiology, Biotechnology and Food Sciences*, *14*(4), Article e11913. https://doi.org/10.55251/jmbfs.11913

Al-Bderee, N. M. H., Al-Janabi, N., Al-Saad, N. F. N., Al-Mousawi, H. T. M., & Abbas, M. D. (2024). Enterobacter cloacae lipopolysaccharide export system protein (lptC) gene expression variation via exposure to biosynthesized zinc oxide nanoparticles. *Journal of Microbiology, Biotechnology and Food Sciences*, *14*(2), Article e10785. https://doi.org/10.55251/jmbfs.10785

Candel, F. J., González Del Castillo, J., Julián Jiménez, A., & Matesanz, M. (2022). Ceftolozane-tazobactam in nosocomial pneumonia. *Revista Española de Quimioterapia*, *35*(Suppl. 1), 35-39. https://doi.org/10.37201/req/s01.08.2022

Chen, X., Liu, H., Liu, S., & Mao, J. (2024). Impact of bacteriocins on multidrug-resistant bacteria and their application in aquaculture disease prevention and control. *Reviews in Aquaculture*, *16*(3), 1286-1307. https://doi.org/10.1111/raq.12897

Clinical and Laboratory Standards Institute. (2022). *Performance standards for antimicrobial susceptibility testing* (32nd ed.; CLSI supplement M100). Clinical and Laboratory Standards Institute.

Collins, J. A., & Osheroff, N. (2024). Gyrase and topoisomerase IV: Recycling old targets for new antibacterials to combat fluoroquinolone resistance. *ACS Infectious Diseases*, *10*(4), 1097-1115. https://doi.org/10.1021/acsinfecdis.4c00128

Dabhi, M., Patel, R., Shah, V., Soni, R., Saraf, M., Rawal, R., & Goswami, D. (2024). Penicillin-binding proteins: The master builders and breakers of bacterial cell walls and its interaction with β-lactam antibiotics. *Journal of Proteins and Proteomics*, *15*(2), 215-232. https://doi.org/10.1007/s42485-024-00135-x

Elshobary, M. E., Badawy, N. K., Ashraf, Y., Zatioun, A. A., Masriya, H. H., Ammar, M. M., Mohamed, N. A., Mourad, S., & Assy, A. M. (2025). Combating antibiotic resistance: Mechanisms, multidrug-resistant pathogens, and novel therapeutic approaches: An updated review. *Pharmaceuticals*, *18*(3), Article 402. https://doi.org/10.3390/ph18030402

Gálvez-Benítez, L., de la Rosa, J. M. O., Rodriguez-Villodres, A., Casimiro-Soriguer, C. S., Molina-Panadero, I., Alvarez-Marín, R., Bonnin, R. A., Naas, T., Pachón, J., Cisneros, J. M., Lepe, J. A., & Smani, Y. (2023). Role of blaTEM and OmpC in the piperacillin-tazobactam resistance evolution by E. coli in patients with complicated intra-abdominal infection. *Journal of Infection*, *87*(3), 220-229. https://doi.org/10.1016/j.jinf.2023.07.005

Gonçalves, R. M., Monges, B. E. D., Oshiro, K. G. N., Cândido, E. S., Pimentel, J. P. F., Franco, O. L., & Cardoso, M. H. (2025). Advantages and challenges of using antimicrobial peptides in synergism with antibiotics for treating multidrug-resistant bacteria. *ACS Infectious Diseases*, *11*(2), 323-334. https://doi.org/10.1021/acsinfecdis.4c00702

Han, Y., Zhang, M., Lai, R., & Zhang, Z. (2021). Chemical modifications to increase the therapeutic potential of antimicrobial peptides. *Peptides*, *146*, Article 170666. https://doi.org/10.1016/j.peptides.2021.170666

Hong, H. W., Kim, Y. D., Jang, J., Kim, M. S., Song, M., & Myung, H. (2022). Combination effect of engineered endolysin EC340 with antibiotics. *Frontiers in Microbiology*, *13*, Article 821936. https://doi.org/10.3389/fmicb.2022.821936

Jassim, Y. A., Jassim, Z. M., Radhi, S. H., & Abed, A. S. (2024). Isolation and diagnosis of Acinetobacter baumannii that produce protease and antimicrobial agents from the milk factory. *Nativa*, *12*(2), 397-402. https://doi.org/10.31413/nat.v12i2.17392

Jawad, A. H. M., Hashim, A. B. N. M., & Abdul-Husin, I. F. (2024). Gene expression variation of blaTEM and blaOXA genes in pathogenic bacteria under colicin effect. *Korean Journal of Microbiology*, *60*(3), 152-160. https://doi.org/10.7845/kjm.2024.4059

Macesic, N., Uhlemann, A. C., & Peleg, A. Y. (2025). Multidrug-resistant Gram-negative bacterial infections. *The Lancet*, *405*(10474), 257-272. https://doi.org/10.1016/S0140-6736(24)02081-6

Moradi, M., Montazeri, E. A., Rafiei Asl, S., Pormohammad, A., Farshadzadeh, Z., Dayer, D., & Turner, R. J. (2025). In vitro and in vivo antibacterial and antibiofilm activity of zinc sulfate ($ZnSO_4$) and carvacrol (CV) alone and in combination with antibiotics against

Pseudomonas aeruginosa. *Antibiotics*, *14*(4), Article 367. https://doi.org/10.3390/antibiotics14040367

Ngo, T. T. T., Nguyen, T. A. T., Huynh, T. Q., & Nguyen, T. T. H. (2024). Assessment of the synergistic effects of ciprofloxacin and ceftazidime combination on *Pseudomonas aeruginosa*. In *International Conference on the Development of Biomedical Engineering in Vietnam* (pp. 960-971). Springer. https://doi.org/10.1007/978-3-031-90197-3_76

Obaid, R. H., Hashim, N. M., & Abbas, M. D. (2025). Enhanced antimicrobial efficacy of leucine-ciprofloxacin-ceftazidime combination against MDR *E. coli*. *SAR Journal of Pathology and Microbiology*, *6*(4), 166-172. https://doi.org/10.36346/sarjpm.2025.v06i04.004

Ojaimi, R. R., & Shliouh, N. H. (2025). Chemical synthesis, characterization, and antibacterial activity of copper oxide nanoparticles: In vitro study on uropathogenic bacteria. *Biomedical and Biotechnology Research Journal*, *9*(3), 264-268. https://doi.org/10.4103/bbrj.bbrj_198_25

Thummeepak, R., Kitti, T., Kunthalert, D., & Sitthisak, S. (2016). Enhanced antibacterial activity of *Acinetobacter baumannii* bacteriophage ØABP-01 endolysin (LysABP-01) in combination with colistin. *Frontiers in Microbiology*, *7*, Article 1402. https://doi.org/10.3389/fmicb.2016.01402

Wang, J., Lu, X., Wang, C., Yue, Y., Wei, B., Zhang, H., Wang, H., & Chen, J. (2024). Research progress on the combination of quorum-sensing inhibitors and antibiotics against bacterial resistance. *Molecules*, *29*(7), Article 1674. https://doi.org/10.3390/molecules29071674

Xiao, G., Li, J., & Sun, Z. (2023). The combination of antibiotic and non-antibiotic compounds improves antibiotic efficacy against multidrug-resistant bacteria. *International Journal of Molecular Sciences*, *24*(20), Article 15493. https://doi.org/10.3390/ijms242015493

Zhu, D.-D., Li, X.-R., Ma, T.-F., Chen, J.-Q., Ge, C.-H., Yang, S.-H., Zhang, W., Chen, J., Zhang, J.-J., Qi, M.-M., Zhang, L., & Yang, H.-J. (2025). Multidrug-resistant extraintestinal pathogenic *Escherichia coli* exhibits high virulence in calf herds: A case report. *Microbiology Research*, *16*(3), Article 59. https://doi.org/10.3390/microbiolres16030059

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توصیف فنوتیپی و مولکولی سویه‌های چنددارویی مقاوم اشریشیا کلی حامل ژن‌های مقاومت جداشده از موارد بالینی مختلف در عراق

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چکیده

هدف: اشریشیا کلی مقاوم به چند دارو (MDR) از مهم‌ترین عوامل ایجاد یوروسپسیس به شمار می‌رود و بار اقتصادی قابل‌توجهی را بر بیمارستان‌ها در سراسر جهان تحمیل می‌کند. گسترش سریع مقاومت چنددارویی در جمعیت‌های E. coli درمان عفونت‌ها را دشوارتر و پرهزینه‌تر ساخته و نیاز به رویکردهای درمانی جدید را افزایش داده است. هدف این مطالعه، بررسی ویژگی‌های فنوتیپی و مولکولی مقاومت چنددارویی در سویه‌های E. coli جداشده از بیماران عراقی مبتلا به عفونت‌های مجاری ادراری (UTIs) و عفونت زخم بود.

مواد و روش‌ها: در مجموع ۳۰ نمونه بالینی از ادرار میانی (MSU) و ترشحات زخم جمع‌آوری شد. شناسایی فنوتیپی E. coli بر اساس شکل، اندازه، بافت و رنگ کلنی‌ها روی محیط کشت انجام و با استفاده از سیستم VITEK 2 Compact تأیید شد. از

میان آن‌ها، ۱۰ سویه به‌عنوان MDR تأیید شدند که ۱۰۰٪ مقاومت نسبت به سیپروفلوکساسین (CIP) و سفتازیدیم (CAZ) نشان دادند. تعیین حداقل غلظت مهاری (MIC) با روش ریزرقیق‌سازی چکربورد انجام شد و شاخص غلظت مهاری کسری (FIC) محاسبه گردید. بررسی بیان ژن‌های emrD و marA با روش RT-qPCR انجام شد. تحلیل آماری داده‌ها با نرم‌افزارهای SPSS نسخه ۲۷ و GraphPad Prism نسخه ۱۰ صورت گرفت.

نتایج: ترکیب CIP و CAZ میزان MIC را از ۲۰۰ و ۲۶۰ میکروگرم بر میلی‌لیتر (به‌صورت جداگانه) به ۵۰ میکروگرم بر میلی‌لیتر (به‌صورت ترکیبی) کاهش داد و شاخص ΣFIC برابر با ۰/۴۹۹۵ نشان‌دهنده اثر هم‌افزایی قوی بود. ترکیب آنتی‌بیوتیک‌ها (CIP و CAZ) با باکتریوسین‌ها و لوسین فعالیت ضدباکتریایی بیشتری ایجاد کرد، به‌ویژه در غلظت‌های ۵۰ تا ۶۰ میکروگرم بر میلی‌لیتر که با کاهش معنی‌دار نرخ رشد باکتری ($P < 0.01$) همراه بود. بررسی بیان ژنی نشان داد که در تمامی درمان‌های ترکیبی، کاهش قابل‌توجهی در بیان ژن‌های emrD و marA رخ داده است. بیان ژن emrD از ۱/۰۶±/۳۶ (کنترل) به ۰/۰۵±۰/۰۵ (CIP/leucine) کاهش یافت و بیان ژن marA از ۱/۱۷±۰/۴۷ به ۰/۰۳±۰/۰۲ (CAZ/bacteriocin) رسید. برای همه مقادیر $P < 0.01$ بود.

نتیجه‌گیری: نتایج این مطالعه از به‌کارگیری ترکیبات آنتی‌بیوتیک-کمکی به‌عنوان راهبردی امیدبخش برای مقابله با عفونت‌های ناشی از E. coli مقاوم به چند دارو حمایت می‌کند. این یافته‌ها با مطالعات جهانی درباره هم‌افزایی ضدمیکروبی همسو بوده و اهداف ژنی جدیدی را برای کنترل تغییرات مقاومت معرفی می‌کند.

کلمات کلیدی: ترکیب آنتی‌بیوتیکی، سفتازیدیم، سیپروفلوکساسین، emrD، marA

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