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THE MOLECULAR EFFECTS OF BIOSYNTHESIZED COLICIN ON PATHOGENIC BACTERIA

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ABSTRACT

Infectious diseases caused by pathogenic bacteria are recognized worldwide as a significant and pressing concern for human health. The misuse and overuse of antibiotics have further exacerbated the issue by giving rise to the emergence of multidrug-resistant bacteria, rendering infections caused by these strains virtually untreatable. The objective of the study was to assess colicin's capability to prevent the growth of multidrug-resistant (MDR) pathogenic bacteria. Forty *E. coli* samples were obtained and subjected to diagnostic and confirmatory tests to verify their purity and ability to produce colicin. Isolates producing colicin were identified using the agar plate diffusion method. Colicin was subsequently isolated, purified, and its approximate molecular weight was determined. The activity of colicin was then assessed on 10 antibiotic-resistant pathogenic bacteria, resulting in different inhibition rates. The gene expression of *blaCTX* and *blaSHV* genes was determined using RT-qPCR analysis, revealing significant effects on the gene expression level. The responses of *blaCTX* and *blaSHV* genes to colicin treatment were found to be significant. The impact of colicin varied among pathogenic bacterial isolates, with some showing a strong response to colicin treatment, while others did not exhibit significant differences after treatment. In summary, the findings of this study suggested that the colicin protein in *E. coli* has the potential to be an alternative to traditional antibiotics for addressing antibiotic-resistant pathogenic bacteria.

Keywords: *E. coli Synthesis, colicin, RT-qPCR, blaCTX, blaSHV*

INTRODUCTION

Colicins are a subgroup of bacteriocins, stable, toxic peptides produced by several *Escherichia coli* strains. Bacteriocin biosynthesis employs some genetic and biochemical pathways **(Choi *et al.*, 2023)**. Colicins exhibit antibacterial activity, degrading the cytoplasmic membrane of permissive cells and binding to specific receptors in both the cytoplasm and membrane, and some exhibit nuclease activity. Studies carried out in *E. coli* have shown that these colicins are associated with several genetic elements. The view that has emerged is that colicins have the property to bind to a diverse set of biological "molecular targets" with high affinity, and once bound, act on the respective molecular cellular processes in a manner analogous to an anti-metabolite drug having multiple modes of action **(Bach *et al.*, 2022)**. Research on colicins has increased appreciation of the possible roles these natural substances have in the microbial ecosystem, and it is suggested that some may be growth or virulence factors or involved in bacterial communication **(Marković *et al.*, 2022)**. The ability of a large fraction of *E. coli* to produce colicins, which the bacteria are can subsequently able to release into their environment, suggests that the bacteria exist in a complicated and multifaceted niche full of bacterial competitors. Although the detailed study of colicins as antimicrobials has a history that goes back over 80 years, it was in the mid-twentieth century that these substances and colicin research began to evolve **(Jin *et al.*, 2021)**.

The colicins are small, cationic proteins that are synthesized by some *E. coli* strains to kill closely related "self" strains or sanitize the gastrointestinal tract of animals of foreign-invasive "non-self" strains/types. Colicins kill cells by many different mechanisms they have evolved **(Liu *et al.*, 2023)**. They can be divided into two broad categories based on their mode of action: the pore-forming colicins that attack the cytoplasmic membrane and colicins that kill cells by disrupting key cellular processes occurring in the cytoplasm. The mechanisms of action of colicins are defined by the subject of their destructive activity. There are two main mechanisms of how colicins evince their antimicrobial effects on target bacteria. The first of these involves the colicin entering the cell. This can happen via spontaneous translocation of colicin through the cell wall, causing a buildup of a colicin channel for import into the cell. This channel allows for the subsequent passage of other colicin molecules to achieve the killing of the targeted cell. Such a mechanism is used by a variety of colicins. The second mechanism involves the binding of the colicin to a cell surface receptor, followed by activation leading to the colicin's translocation into the cell **(Calcuttawala *et al.*, 2022)**.

Once inside the target bacterium, most colicins will interfere with some essential cellular functions to inhibit cell growth. Many colicins have multiple intracellular targets, which accounts for their wide spectrum of action. For instance, colicin E2 targets and blocks the bacterial Mg^{2+} uptake protein, followed by cell killing through the activation of the RNase E coded structure **(Meade *et al.*, 2020)**. The colicins' bactericidal actions are highly specific, which is a feature associated with the colicins' receptor binding mode. Other than growth arrest, colicins may mediate either cell lysis or a more regulated apoptotic cell death. This means that colicins can facilitate the competitive exclusion of other competing strains in their colicin-producing environment. All these mechanisms and insights into these processes are particularly pertinent in the context of multi-drug resistant strains of bacteria. The first step for assessing their potential as clinical candidates or an alternative to current antibiotics is to understand colicin biology **(Parker & Davies, 2022)**. This understanding can be used to help dispel some concerning aspects of colicin production, such as the incompatibility due to amino acid specificity, by making bacteria more manipulatable. In addition, knowledge of colicin production can be used as leverage to help improving their antibacterial activity, for instance, by modifying their structure in a way that bolsters killing in multi-drug resistant strains **(Mihaylova *et al.*, 2024)**.

The global spread of infections caused by multidrug-resistant (MDR) pathogens has re-emphasized the urgent need for new antibiotics. Colicins are considered an adjunct to antibiotics to treat MDR infections. Colicins comprise a cocktail of narrow-spectrum antibacterial proteins and only target few strains of a single bacterial species, thus sparing the beneficial microbiota and minimizing collateral damage. Their ability to kill pathogens at low concentrations could limit the chance of developing resistance during anti-infective treatment or to be used in combination therapy **(Al-Bderee *et al.*, 2024)**. The identification of a number of potential clinical scenarios where colicin therapy may be beneficial includes severe infections where anti-infective treatment options are limited or exhausted; chronic infection exacerbations caused by the same strain of bacteria and likely caused by biofilm structures; and long-term treatment strategies for those with frequent infection exacerbations caused by the same bacterial strain, especially cystic fibrosis (CF) patients **(Abady *et al.*, 2017)**.

The extensive utilization of antibiotics guarantees the persistence of multidrug-resistant pathogens and the continued emergence of new ones. Reversing antimicrobial resistance is challenging, and there is a crucial requirement for new antimicrobials that are effective against these hard-to-treat multidrug-resistant pathogens **(Abdul-Husin *et al.*, 2016)**. It is anticipated that antibiotic resistance will outpace the development of new treatment options, necessitating a deeper comprehension of the molecular, evolutionary, and ecological mechanisms of antibiotic resistance spread **(Deyno *et al.*, 2017)**. To tackle the current antimicrobial resistance crisis, entirely new strategies must be devised to combat these pathogens, such as the combination of antimicrobial drugs with other agents that counteract antibiotic-resistant mechanisms **(Akgul *et al.*, 2017)**. Moreover, a more personalized approach based on precise diagnostic tools will ensure prompt and targeted treatments, as many antibiotics are frequently prescribed inappropriately. Additionally, better control is necessary for the overall use and release of antibiotics into the environment **(Medina and Pieper, 2016)**.

A plausible way of treating individuals whose infections have become untreatable is to use a cocktail of proven colicins that all target the same strain. Although some obstacles will need to be overcome, such as how to refine dosing, identifying candidates where colicin therapy is most likely to succeed, how best such tailored treatments are manufactured and delivered, addressing potential resistance mechanisms, and appreciating the exact long-term effect of colicin use on the wider flora that stands in the way of colicin clinical use, these are surmountable. However, these efforts must be underpinned with evidence from high-quality clinical trials if the ultimate goal of a licensed colicin-based therapy for MDR bacteria is to be achieved **(Chikindas *et al.*, 2018)**.

In this research, we aim is to explore alternative solutions to antibiotics and focus on inhibiting pathogenic bacteria using naturally derived products. We will examine the colicin production for various *E. coli* isolates and assess its effectiveness in inhibiting or eradicating multidrug-resistant bacteria. Additionally, we will investigate the variation in gene expression of certain virulence factors, specifically the *blaCTX* and *blaSHV* genes, across different isolates.

MATERIAL AND METHODS

Sample collection

Samples from various locations were gathered using sterilized swabs **(Naser *et al.*, 2024)**. A total of 40 *E. coli* samples were collected from various sources to study colicin production. For pathogenic isolates, 30 samples were acquired from urinary tract infection cases, 25 from wound injuries, and 20 from foot ulcer patients. All the isolates were retrieved from stored cultures in the Microbiology Laboratory of the Department of Medical Biotechnology at Al-Qasim Green University, ensuring no direct contact with the patients. The identification of all isolated bacteria was carried out through phenotypic appearance and biochemical tests, with confirmation of the diagnosis using the Vitek2 Compact System.

Colicin production and purification steps

The production of colicin involves isolating and identifying the producing bacterial isolate, as well as the processes utilized in overproducing and purifying colicin, following the techniques outlined in the previously published article, **Jawad *et al.*, 2024**.

Shortly, the research aimed to identify strains that produce colicin using Mueller-Hinton agar or Nutrient agar. The isolates were assessed using the agar-well method, and the diameter of inhibition zones was measured in millimeters. *E. coli* and *Klebsiella* strains were used as controls to evaluate colicin production. The expansion and activation of colicin-producing bacteria involved introducing 20 ml of *E. coli* from Brain Heart Broth into 1L of Brain Heart Broth prepared with mitomycin-C under sterile conditions. The resulting solution was placed in a shaking incubator at 37 ºC for 48 hours and then centrifuged at 14,000 rpm for 15 minutes to separate the sediment from the filtrate. The filtrate was stored at refrigeration temperature. Ammonium sulfate was used for the extraction and precipitation of colicin at 70% saturation within a temperature ranged from 0-4°C. The residue was collected using a refrigerated centrifuge at 3000 rpm, and the sample was repeated twice. After precipitation, dialysis was performed to eliminate contaminants, and ultrafiltration with a size range of 50-100 Daltons was performed to ensure the absence of other proteins. The molecular weight and purity of the extracted colicin were determined using SDS-polyacrylamide electrophoresis.

Antibacterial activity of purified colicin

The effectiveness of colicin against bacteria was tested using a serial dilution method similar to the minimum inhibitory concentration (MIC) technique used for assessing antibiotics **(Alasady *et al.*, 2021)**. This process involved diluting colicin produced by *E. coli* and applying it to various pathogenic isolates using the McFarland method on Mueller Hinton agar plates. To conduct the test, sterile petri dishes were filled with 250 ml of Mueller Hinton agar medium, and the bacterial sample was evenly spread on the plate using a sterile cotton swab. Wells of 6 mm diameter were made in the agar for each concentration—62, 125, 250, 500, and 1000 µg/mL, with one well reserved for the control group (D.W as the control) using a cork borer. The plates were then incubated for 24 hours at 36±1 oC under aerobic conditions, resulting in the observation of confluent bacterial growth after incubation. The inhibition of bacterial growth was measured in millimeters. A time-kill kinetics test was used to assess the impact of colicin exposure in the cultures. For the test, new cultures were grown in colicin-containing medium, while for the control; other cultures were grown without the addition of colicin. The highest dilution that created an inhibition zone indicated the strength of colicin's activity **(Hindi *et al.*, 2017)**. Therefore, the activity of colicin is directly related to the reciprocal of the highest dilution factor that produces a detectable zone of inhibition. The MIC concentration of colicin was determined to be 125 µg/mL.

Gene expression for *blaCTX* and *blaSHV* in MDR bacteria under colicin effect

The study examined the impact of colicin on the expression of the (*blaSHV*) gene in selected isolates; *Salmonella*, *Klebsiella*, and *Pseudomonas*, and for the (*blaCTX*) gene the selected isolates; *E. coli*, *Proteus*, *Acentobacter*, and *Strepococcus* by determining the inhibition zone at a colicin concentration of 125 µg/ml based on MIC value. Molecular expression was analyzed using RT-qPCR acoording to **Lu *et al.*, 2024**. All isolates had their total RNA extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) as per the modified manufacturer's instructions, and Fermentas, USA's DNase was used to eliminate genomic DNA from the isolated RNA. The purity of the RNA was assessed using formaldehyde-denaturing 1.2% (w/v) agarose gel electrophoresis, and the quantities and absorbance ratios of RNA at A260/A280 and A260/A230 were determined using the Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). The total RNA (0.5 µg) was converted into single-stranded cDNA using Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase and random hexamer oligonucleotides (Fermentas, USA) according to the manufacturer's instructions. The generated cDNA was used to amplify the *blaSHV* and *blaCTX* genes with the primer sequences listed in Table (1). Real-time PCR was performed using ™SYBR Green qPCR Master Mix (Fermentas, USA) and a Bio-Rad MiniOpticonTM equipment (USA) with amplification conditions listed in Table (2). The expression levels of the *blaSHV* and *blaCTX* genes were determined using the comparative Ct technique ($2^{-\Delta Ct}$ formula) following normalization with the 16S rRNA gene **(Kadhim *et al.*, 2024)**.

Table 1 PCR primers used in the study

Primer names	Primer Sequences (5'-3')	Product Size (bp)	Source of primer
***Housekeeping 16S rRNA* gene**	F-(5' -AGAGTTTGATCATGGCTCAG-3') R-(5' -GCTGCCTCCCGTAGGAGT-3')	355 bp	This study
***blaSHV* gene**	F-(5'- AGGATTGACTGCCTTTTTG -3') R-(5'- ATTTGCTGATTTCGCTCG -3')	392 bp	(Khalil *et al.*, 2020)
***blaCTX-M* gene**	F-(5' – CCGCTGCCGGTYTTATC - 3′) R-(5′ - ATGTGCAGYACCAGTAA- 3′)	504 bp	(Cottell *et al*., 2013)

Table 2 qPCR conditions for *16S rRNA*, *blaSHV* and *blaCTX* genes for all bacteria isolate subjected in this study.

Primer	Initial Denaturation	Cycling condition			Final Extension	Number cycle
		Denaturation	Annealing	Extension		
16S rRNA	95 ºC for 3 min	95 ºC for 10 sec	53 ºC for 30 sec	72 ºC for 1 min	72 ºC for 10 min	40
blaSHV	95 ºC for 3 min	95 ºC for 10 sec	57 ºC for 30 sec	72 ºC for 1 min	72 ºC for 10 min	40
blaCTX	95 ºC for 3 min	95 ºC for 10 sec	58 ºC for 30 sec	72 ºC for 1 min	72 ºC for 10 min	40

Statistical Analysis

The impact of various factors on the original study characteristics was analyzed using the SAS program for statistical analysis. To assess the means significantly, the Least Significant Difference (Dmt) test of ANOVA was employed. The study involved a meaningful comparison of percentages (0.05 and 0.01 likelihood) using the Chung test **(Ali, Z., & Bhaskar, S. B., 2016)**.

RESULTS AND DISCUSSION

Identification of bacterial isolates

The identification of all the isolated bacteria was based on their phenotypic characteristics and biochemical tests, and the diagnosis was confirmed using the Vitek2 Compact System. Among the 40 isolates, only two *E. coli* strains were found to be colicin producers. On the other hand, 20% of the *E. coli* isolates were pathogenic, along with 7% of *Salmonella*, 11% of *Klebsiella*, 10% of *Pseudomonas*, 7% of *Staph*. *Epiderma*, 13% of *Proteus*, 7% of *Enterococcus faecalis*, 7% of *Acinetobacter baumannii*, 9% of *Staphylococcus aureus*, and 9% of *Strepococcus*.

Investigation of colicin producing *E. coli* isolate

The investigation into colicin production from 40 *E. coli* isolates involved using the agar well diffusion method, where the diameters of the inhibition zones around the holes were measured in millimeters. From the results, it was observed that only one of the isolates (no. 16) displayed inhibitory activity against other bacteria (*E. coli* from another genus and *Proteus*), as depicted in Figure (1). This particular isolate exhibited 20 mm inhibition for cultured *E. coli* and 23 mm inhibition for cultured *Proteus*. Based on the aforementioned data, it is apparent that the solid medium encourages the antagonistic relationship between the two isolates and supports the growth and spread of colicin within the medium.

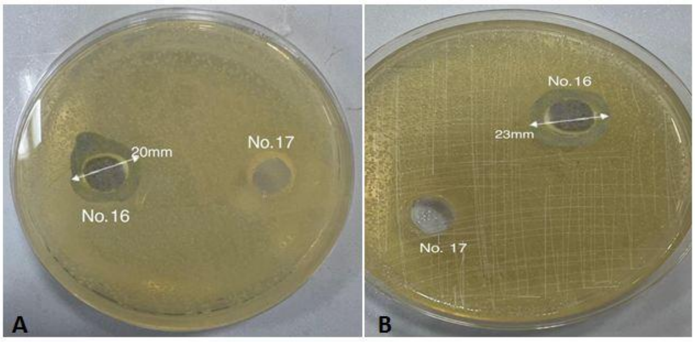

Figure 1 Colicin obtained from isolate *E. coli* No. 16 and tested against test bacteria, using the Agar Well Diffusion Method (The well contains 100µl of *E. coli*). A- The plate cultured with Escherichia *coli* of a different genus, B- The plate cultured with *Proteus*.

Activity of colicin on pathogenic bacterial isolate

The effectiveness of colicin against various pathogenic bacteria, including both Gram-positive (such as *Staphylococcus aureus*, *Streptococcus*, *Enterococcus faecalis*, and *Staphylococcus epidermidis*) and Gram-negative (such as *E. coli*, *salmonella*, *Proteus*, *Acinetobacter baumanni*, *Klebsiella*, and *Pseudomonas*) bacteria, has been demonstrated. Using the agar well fusion method, colicin concentrations resulted in inhibition zone diameters in different ranges when tested on pathogenic bacterial isolates. The results were obtained through the dilution method, employing concentrations of 1000 µg/ml, 500 µg/ml, 250 µg/ml, and 125 µg/ml prepared from colicin, as shown in Figure (2).

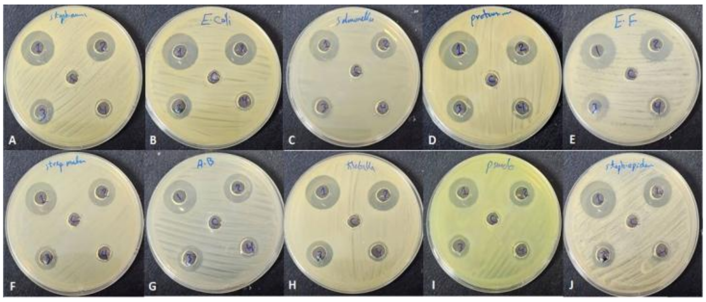

Figure 2 Illustrating inhibition zones of colicin-producing *E.coli* against gram positive pathogenic bacteria, A- *Staphylococcus aureus*, B- *E coli*, C- *Salmonella*, D- *Proteus*, E- *Enterococcus faecalis*, F- *Streptococcus*, G- *Acinetobacter baumanni*, H- *Klebsiella*, I- *Pseudomonas*, J- *Staphylococcus epidermidis* where the numbers (1, 2, 3 and 4) represent the concentrations of colicin (1000 µg/ml, 500 µg/ml, 250 µg/ml and 125 µg/ml) respectively, C: Control

Gene expression of *blaSHV* and *blaCTX* genes of pathogenic bacteria under effect of colicin

The fold change in gene expression was determined for all selected bacterial isolates using the ($2^{-\Delta Ct}$ formula). The P value was consistently significant across all isolates. Table (3) and Table (4) demonstrate the high significance of the P value for the majority of pathogenic bacteria, indicating the impact of colicin on the gene expression folding rate for the *blaCTX* and *blaSHV* genes, resulting in decreased folding rates in most bacterial isolates after colicin treatment compared to before treatment, as illustrated in Figure (3) and Figure (4) respectively.

Table 3 The expression of the *blaCTX* gene in five chosen pathogenic bacteria was compared between isolates treated with colicin and untreated isolates to calculate the fold change.

Type of Isolate	**Treatment colicin**	*Ct of target gene (blaCTX)*	*Ct of reference gene (16S rRNA)*	*ΔCt of CTX*	*ΔΔCt of CTX*	*Fold of gene expression*	*P value*
Proteus	Untreated	21.59	22.03	-0.44	-1.51	2.85	≤ 0.001**
	Treated	26.01	22.11	3.90	2.83	0.14	
E.coli	Untreated	17.39	16.15	1.24	0.17	0.89	≤ 0.001**
	Treated	21.15	16.25	4.90	3.83	0.07	
Streptococcus	Untreated	22.24	19.82	2.42	1.35	0.39	0.017*
	Treated	24.11	19.93	4.18	3.11	0.12	
Acinetobacter	Untreated	21.52	18.72	2.80	1.73	0.30	0.010*
	Treated	24.02	18.85	5.17	4.10	0.06	
***P*≤ 0.05**							

Table 4 The expression of the *blaSHV* gene in five chosen pathogenic bacteria was compared between isolates treated with colicin and untreated isolates to calculate the fold change.

Type of Isolate	**Treatment colicin**	*Ct of target gene (blaSHV)*	*Ct of reference gene (16S rRNA)*	*ΔCt of SHV*	*ΔΔCt of SHV*	*Fold of gene expression*	*P value*
Pseudomonas	Untreated	20.03	22.11	-2.08	-2.01	4.03	≤ 0.001**
	Treated	25.39	22.20	3.19	3.26	0.1	
Salmonella	Untreated	20.70	17.12	3.58	3.65	0.08	1
	Treated	20.73	17.25	3.48	3.55	0.09	
Klebsiella	Untreated	14.18	15.89	-1.71	-1.64	3.12	≤ 0.001**
	Treated	19.88	15.93	3.95	4.02	0.06	
***P*≤ 0.05**							

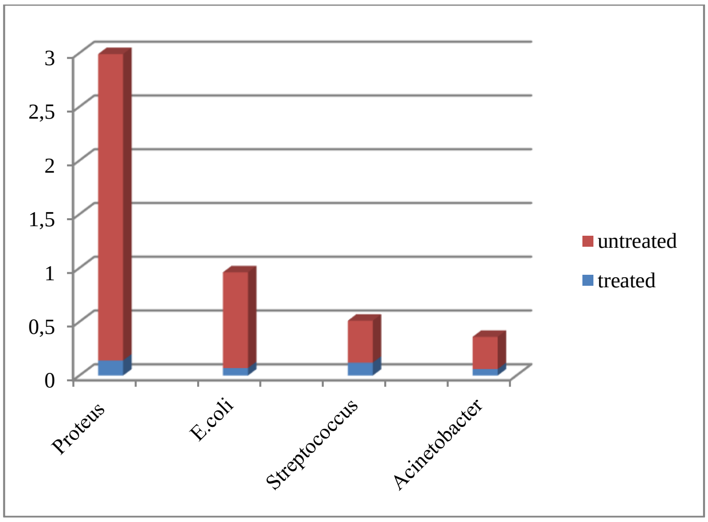

Figure 3 The results display a variation in *blaCTX* gene expression, indicating a difference in gene expression ($2^{-\Delta\Delta C\ T}$) values between the treated and untreated samples. The difference is statistically significant with a p-value of less than 0.05.

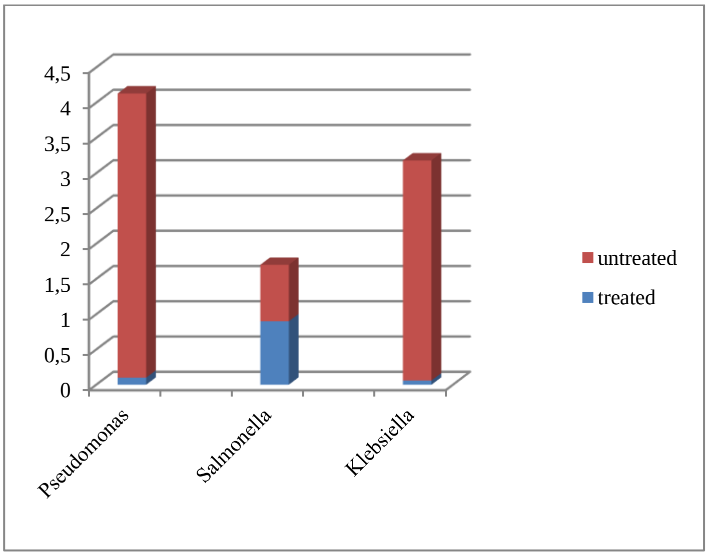

Figure 4 The results display a variation in *blaSHV* gene expression, indicating a difference in gene expression ($2^{-\Delta\Delta C\ T}$) values between the treated and untreated samples. The difference is statistically significant with a p-value of less than 0.05.

DISCUSSION

Colicins are proteins secreted by some bacteria and are lethal to the same or closely related species. The molecular mechanism is to bind to specific receptor proteins of sensitive bacteria to form complexes, thereby destroying the cell membrane and inhibiting the growth of bacteria (**Fokt *et al.*, 2022**). The focus of this research was to determine the ability of *E. coli* to produce colicin protein and use it for the treatment of bacterial infectious diseases. Many studies have tested the level of colicin production by *E. coli* when exposed to mitomycin-C. Colicin-producing strains have also been proposed to use colicins to restrict the growth of other enteric organisms and thus prevent them from reducing the host's food supply. In the same vein, other similar bacteriocins could also have this application where food preservation is compromised by entropy-resistant strains. Furthermore, the susceptibility of MDR enterohemorrhagic *E. coli* to colicins indicates that a greater study of the biochemistry of colicins could suggest a novel strategy for biobase agents with anti-MDR EHEC (**Jadooa *et al.*, 2024**). It is clear from their many mechanisms of action that colicins can serve to destroy a member of the population within an ecosystem. However, it has been proposed that they can also play a preventative role by serving as a competitive inhibitor to prevent the establishment of other susceptible bacteria. It was later verified that at sufficiently low concentrations, colicin can prevent susceptible bacteria from establishing themselves in a culture or in competition with other bacteria. This was, at first, presumed to be due to their mode of action, as the cells are vulnerable to destruction. Thus, the "antagonistic" activities of colicins could involve not only the killing activity by colicin but also the anti-termination capability of the E colicin. Indeed, the differential killing efficiencies of colicins suggest that the many other diverse mechanisms of colicin action must be important if the same col plasmid can evolve the same specific immunity (**Francis, Webby *et al.*, 2021**).

This study revealed that not all *E. coli* isolates are capable of producing colicin, indicating that the genes responsible for the production of colicine are carried on the plasmid rather than the bacterial DNA material. Furthermore, colicin production only occurs under specific and unique conditions, which may aid the bacteria in surviving and thriving in challenging environments or in outcompeting others for nutrients (**Jawad *et al.*, 2024**). The study's findings demonstrate that increasing the concentration of colicin leads to greater inhibition of multidrug-resistant pathogenic bacteria.

The impact of colicin on pathogenic bacteria varied depending on the level of *blaSHV* gene expression in *Salmonella*, *Klebsiella*, and *Pseudomonas*. Similarly, the level of *blaCTX* gene expression in *E. coli*, *Proteus*, *Acentobacter*, and *Strepococcus* differed for each isolate. The effectiveness of colicin in fighting MDR bacteria is interesting because it is known to be effective against closely related species. **Ares-Arroyo *et al.*, 2021**, discovered that colicin produced by *E. coli* bacteria successfully combated pathogens such as *Staphylococcus aureus*, *Pseudomonas fluorescens*, *P. aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, *Listeria monocytogenes*, *E. coli* O157:H7, and *Clostridium botulinum*.

According to **Charkhian *et al.*, 2024**, colicin may have a broad spectrum of activity against a wide range of related or unrelated species. It would be valuable to further investigate the use of colicin against other bacterial species in the future. In the research by **Widodo *et al.*, 2023**, it was observed that all indicator organisms were susceptible to colicin but resistant to certain antibiotics in the comparative antibiotic sensitivity test. **Al-mawlawi & Obaid, 2017**, found that colicin-producing *E. coli* strains exhibited more resistance to tetracycline, neomycin, and ampicillin compared to non-colicin producing strains. **Łojewska *et al.*, 2020**, further reported that colicin-producing *E. coli* had high levels of antibiotic resistance. Many studies have provided evidence that specific *E. coli* strains could possess both colicin genes and antibiotic resistance genes (**Obaid Hasson *et al.*, 2024**). **Temikotan, 2023**, indicated that the production of colicin in *E. coli* is associated with the presence of a colicogenic plasmid complex. This supports the fact that the trait of colicin production is linked with plasmids, and the recognized plasmids are identified as Col-plasmids, each with a molecular weight of 4kb. Colicins are effective alternatives to antibiotics, especially because they interfere with certain components of bacterial cells to inhibit growth, unlike many antibiotics such as canamycin, penicillin, and ciprofloxacin that target other activities in bacteria. Additionally, colicins are safe for use in humans, as they only act on bacteria with specific receptors, which are not present in human cells. Therefore, colicins have a rapid action through cell killing and may destroy pathogenic cells during their active growth phase and prevent the development of resistance (**LAMBERTI *et al.*, 2022**).

CONCLUSION

Investigating the potential of colicins while considering the associated risks shows promise in combating antibiotic-resistant pathogens and advancing antimicrobial therapeutics. Emphasizing the necessity for further research into the advantages of bacteriocins over antibiotics is crucial for developing effective solutions to the pressing global health issue of bacterial resistance. The identification of *E. coli* strains that produce colicins and their association with antibiotic resistance is highly significant. The strong correlation observed between colicin production and antibiotic resistance suggests that colicins could function as natural alternatives to traditional antibiotics. Additionally, exploring plasmid-mediated gene transfer adds to our understanding how colicin genes and antibiotic resistance spread. The results of this study have direct implications for public health.

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REFERENCES

Choi, G. H., Holzapfel, W. H., & Todorov, S. D. (2023). Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Critical Reviews in Microbiology*, *49*(5), 578-597. https://doi.org/10.1080/1040841X.2022.2090227

Bach, E., Passaglia, L. M. P., Jiao, J., & Gross, H. (2022). Burkholderia in the genomic era: from taxonomy to the discovery of new antimicrobial secondary metabolites. *Critical Reviews in Microbiology*, *48*(2), 121-160. https://doi.org/10.1080/1040841X.2021.1946009

Marković, K. G., Grujović, M. Ž., Koraćević, M. G., ..., & Djilas, M. D. (2022). Colicins and microcins produced by Enterobacteriaceae: characterization, mode of action, and putative applications. *International Journal of Environmental*

Research and Public Health, *19*(18), 11825. https://doi.org/10.3390/ijerph191811825

Jin, X., An, S., Kightlinger, W., Zhou, J., & Hong, S. H. (2021). Engineering Escherichia coli to produce and secrete colicins for rapid and selective biofilm cell killing. *AIChE journal. American Institute of Chemical Engineers*, *67*(12). https://doi.org/10.1002/aic.17466

Liu, P., Dong, X., Cao, X., Xie, Q., Huang, X., Jiang, J., ... & Luo, K. (2023). Identification of three Campylobacter Lysins and Enhancement of their anti-Escherichia coli efficacy using colicin-based translocation and receptor-binding domain Fusion. *Microbiology spectrum*, *11*(2), e04515-22. https://doi.org/10.1128/spectrum.04515-22

Calcuttawala, F., Pal, A., Nath, P., Kar, R., Hazra, D., & Pal, R. (2022). Structural and functional insights into colicin: a new paradigm in drug discovery. *Archives of Microbiology*, *204*(1), 37. https://doi.org/10.1007/s00203-021-02689-6

Meade, E., Slattery, M. A., & Garvey, M. (2020). Bacteriocins, potent antimicrobial peptides and the fight against multi drug resistant species: resistance is futile?. *Antibiotics*, *9*(1), 32. https://doi.org/10.3390/antibiotics9010032

Parker, J. K., & Davies, B. W. (2022). Microcins reveal natural mechanisms of bacterial manipulation to inform therapeutic development. *Microbiology*, *168*(4), 001175. https://doi.org/10.1099/mic.0.001175

Mihaylova-Garnizova, R., Davidova, S., Hodzhev, Y., & Satchanska, G. (2024). Antimicrobial Peptides Derived from Bacteria: Classification, Sources, and Mechanism of Action against Multidrug-Resistant Bacteria. *Preprints* 2024, 2024080665. https://doi.org/10.20944/preprints202408.0665.v1

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 EXPOSE TO BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES. *Journal of microbiology, biotechnology and food sciences*, *14*(2), e10785-e10785. https://orcid.org/0000-0001-8228-3945

Abady, N. R., Guglielmino, C. J. D., Graham, R. M., Adelskov, J., Smith, H. V., Patel, B. K. C., & Jennison, A. V. (2017). Genetic characterization of a Neisseria meningitidis cluster in Queensland, Australia. *Canadian Journal of Microbiology*, *63*(7), 644-647. https://doi.org/10.1139/cjm-2017-0017

Abdul-Husin, I. F., Hashim, N. M., Hindi, N. K. K., Abood, F. M., & Kadhim, A. K. (2016). Effectiveness of aqueous and alcoholic extract of" Annona Squamosa" plant against some types of Gram positive and negative bacteria. *RESEARCH JOURNAL OF PHARMACEUTICAL BIOLOGICAL AND CHEMICAL SCIENCES*, *7*(6), 294-297.

Deyno, S., Toma, A., Worku, M., & Bekele, M. (2017). Antimicrobial resistance profile of staphylococcus aureus isolates isolated from ear discharges of patients at University of Hawassa comprehensive specialized hospital. *BMC Pharmacology and Toxicology*, *18*, 1-7. https://doi.org/10.1186/s40360-017-0141-x

Akgul, A., Al-Janabi, N., Das, B., Lawrence, M., & Karsi, A. (2017). Small molecules targeting LapB protein prevent Listeria attachment to catfish muscle. *Plos one*, *12*(12), e0189809. https://doi.org/10.1371/journal.pone.0189809

Medina, E., & Pieper, D. H. (2016). Tackling threats and future problems of multidrug-resistant bacteria. *How to overcome the antibiotic crisis: facts, challenges, technologies and future perspectives*, 3-33. https://doi.org/10.1007/82_2016_492

Chikindas, M. L., Weeks, R., Drider, D., Chistyakov, V. A., & Dicks, L. M. (2018). Functions and emerging applications of bacteriocins. *Current opinion in biotechnology*, *49*, 23-28. https://doi.org/10.1016/j.copbio.2017.07.011

Naser, H. H., Kadhim, M. J., & Almhanna, H. (2024). Investigating the impact of the genetic variant CXCR1 (rs2234671) in individuals with urinary tract infections. *Human Antibodies*, (Preprint), 1-10. https://doi.org/10.3233/HAB-230019

Jawad AHM, Hashim ANM, & Abdul-Husin IF (2024). Gene expression variation of *blaTEM* and *blaOXA* genes in pathogenic bacteria under colicin effect. *Korean J. Microbiol*, *60*(3), 152-160. https://doi.org/10.7845/kjm.2024.4059

Alasady, D., Mhadei, K. H., & Naser, M. S. (2021). Evaluation of antimicrobial activity of Commiphora myrrh against standard bacterial strains and clinical isolates with chemical analysis profiling. *Journal of Pharmaceutical Research International*, *33*(47A), 714-723. https://doi.org/10.9734/jpri/2021/v33i47A33066

Hindi, N. K. K., Abdul-Husin, I. F., Al-Mahdi, Z. K. A., Ewadh, R. M., Hossain, A. O., Kadhim, M. J., ... & Al-Yaseri, A. A. (2017). Effectiveness of Aqueous extract of Green, Black and Red Tea Leaves against some types of Gram positive and negative bacteria. *Research Journal of Pharmacy and Technology*, *10*(6), 1957-1962. 10.5958/0974-360X.2017.00343.2

Kadhim, M. J., Al-Janab, H. S. O., Hasson, S. O., & Abbas, Z. M. (2024). INVESTIGATING THE RELATIONSHIP BETWEEN SOME VIRULENCE AND ANTIBIOTICS RESISTANCE GENES OF SOME LOCAL PATHOGENIC BACTERIA IN IRAQ. *Journal of microbiology, biotechnology and food sciences*, *13*(4), e10139-e10139. https://doi.org/10.55251/jmbfs.10139

Lu, J., Abdelhamed, H., Al-Janabi, N., Eissa, N., Lawrence, M., & Karsi, A. (2024). Identification of stable reference genes in Edwardsiella ictaluri for accurate gene expression analysis. *bioRxiv*, 2024-08. https://doi.org/10.1101/2024.08.16.608217

Khalil, O. A., Enbaawy, M. I., Salah, T., Mahmoud, H., & Ragab, E. (2020). In vitro investigation of the antibacterial effect of silver nanoparticles on ESBL-producing E. coli and Klebsiella spp. isolated from Pet animals. *World's Veterinary Journal*, (4), 514-524. https://dx.doi.org/10.54203/scil.2020.wvj62

Cottell, J. L., Kanwar, N., Castillo-Courtade, L., Chalmers, G., Scott, H. M., Norby, B., ... & Boerlin, P. (2013). bla CTX-M-32 on an IncN plasmid in Escherichia coli from beef cattle in the United States. Antimicrobial Agents and Chemotherapy, 57(2), 1096-1097. https://doi.org/10.1128/aac.01750-12

Ali, Z., & Bhaskar, S. B. (2016). Basic statistical tools in research and data analysis. *Indian journal of anaesthesia*, *60*(9), 662-669. https://doi.org/10.4103/0019-5049.190623

Fokt, H., Cleto, S., Oliveira, H., Araújo, D., Castro, J., Cerca, N., ... & Almeida, C. (2022). Bacteriocin production by Escherichia coli during biofilm development. *Foods*, *11*(17), 2652. https://doi.org/10.3390/foods11172652

Jadooa, M. M., Al-Mousawi, H. T. M., & Al-bderee, N. M. H. (2024). Impact of ZnO nanoparticles on MagA gene expression in Klebsiella pneumoniae isolated from diabetic foot ulcers. *Asia Pacific Journal of Molecular Biology and Biotechnology*. https://doi.org/10.35118/apjmbb.2024.032.3.22

Francis, M. L. R., Webby, M. N., Housden, N. G., Kaminska, R., Elliston, E., Chinthammit, B., ... & Kleanthous, C. (2021). Porin threading drives receptor disengagement and establishes active colicin transport through Escherichia coli OmpF. *The EMBO journal*, *40*(21), e108610. https://doi.org/10.15252/embj.2021108610

Ares-Arroyo, M., Rocha, E. P., & Gonzalez-Zorn, B. (2021). Evolution of ColE1-like plasmids across γ-Proteobacteria: From bacteriocin production to antimicrobial resistance. *PLoS Genetics*, *17*(11), e1009919. https://doi.org/10.1371/journal.pgen.1009919

Charkhian, H., Soleimannezhadbari, E., Bodaqlouei, A., Lotfollahi, L., Lotfi, H., Yousefi, N., ... & Gholinejad, Z. (2024). Assessment of bacteriocin production by clinical Pseudomonas aeruginosa isolates and their potential as therapeutic agents. *Microbial Cell Factories*, *23*(1), 175. https://doi.org/10.1186/s12934-024-02450-w

Widodo, A., Lamid, M., Effendi, M. H., Khailrullah, A. R., Kurniawan, S. C., Silaen, O. S. M., ... & Ramandinianto, S. C. (2023). Antimicrobial resistance characteristics of multidrug resistance and extended-spectrum beta-lactamase producing Escherichia coli from several dairy farms in Probolinggo, Indonesia. *Biodiversitas Journal of Biological Diversity*, *24*(1). https://doi.org/10.13057/biodiv/d240126

Al-mawlawi, Z. S., & Obaid, H. H. (2017). Antibacterial Activity of Synergistic Effect of Colicin and Gold Nanoparticles Against Pseudomonas Aerugensa. *Iraqi Journal of Science*, *58*(2C), 1020-1027. https://doi.org/10.24996.ijs.2017.58.2C.4

Łojewska, E., Sakowicz, T., Kowalczyk, A., Konieczka, M., Grzegorczyk, J., Sitarek, P., ... & Kowalczyk, T. (2020). Production of recombinant colicin M in Nicotiana tabacum plants and its antimicrobial activity. *Plant Biotechnology Reports*, *14*, 33-43. https://doi.org/10.1007/s11816-019-00571-y

Obaid Hasson, S., Kamil Hasan, H., Abdul Kadhem Salman, S., Judi, H. K., Akrami, S., Saki, M., ... & Fares Hashem, D. (2024). In vivo and in vitro efficacy of the ithmid kohl/zinc-oxide nanoparticles, ithmid kohl/Aloe vera, and zinc-oxide nanoparticles/Aloe vera for the treatment of bacterial endophthalmitis. *Scientific Reports*, *14*(1), 15746. https://doi.org/10.1038/s41598-024-66341-1

Temikotan, T. (2023). Colicin production, antibacterial effect and molecular sequencing of Escherichia coli isolated form different sources. *Open Access Research Journal of Science and Technology*, *9*(1), 010-020. https://doi.org/10.53022/oarjst.2023.9.1.0055

LAMBERTI, M. F. T., LÓPEZ, F. E., PESCARETTI, M. D. L. M., & DELGADO, M. A. (2022). Characterization of a bacteriocin produced by a clinical isolate of Shigella flexneri 2. *Anais da Academia Brasileira de Ciências*, *94*, e20200982. https://doi.org/10.1590/0001-3765202220200982