

Evaluation of the Effect of Silver Nanoparticles (AgNPs) and Berberine on Expression of *acrB* and *luxS* Genes in Clinical Isolates of *Klebsiella pneumoniae*

Lina Abdulameer S. Alsaadi^{1*}, Ibtihal Hameed Mohsin¹, Zainab Hussein Mahdi²

¹Department of Biology, College of Sciences, University of Diyala, Iraq

²Department of Biology, College of Education for Pure Sciences, University of Diyala, Iraq

Abstract

Klebsiella pneumoniae is a Gram-negative opportunistic pathogen and a major cause of health-care-associated infections worldwide. Between September and December 2025, 200 clinical samples were collected from patients of different ages and genders attending Baquba Teaching Hospital and Al-Batool Teaching Hospital in Diyala, Iraq. Samples were Gram-stained and cultured on blood agar, MacConkey agar, and chromogenic media for *K. pneumoniae*. Of the collected samples, 139 showed positive bacterial growth. The highest frequency of *K. pneumoniae* isolates was detected in urine samples (43.7%), whereas sputum samples showed the lowest frequency (12.5%). Antimicrobial susceptibility testing revealed high resistance to amoxicillin-clavulanic acid (93%), cefotaxime and doxycycline (87%), and trimethoprim-sulfamethoxazole (81%), while the isolates remained relatively sensitive to meropenem (75%) and imipenem (72%). Molecular analysis demonstrated that all isolates (100%) carried the *acrB* and *luxS* genes. Treatment with silver nanoparticles (AgNPs) significantly reduced the expression of both genes, with overall fold-change values of 0.45 for *acrB* and 0.33 for *luxS*, although one isolate (K1) showed a slight increase in *acrB* expression (1.61-fold). Berberine treatment produced a greater reduction in gene expression, with overall fold-change values of 0.20 for *acrB* and 0.29 for *luxS* across all isolates. These findings demonstrate that both AgNPs and Berberine suppress the expression of antibiotic resistance- and quorum sensing-related genes in *K. pneumoniae*, with berberine showing greater inhibitory activity. Therefore, these compounds may serve as promising adjuvants to improve antibiotic efficacy and reduce bacterial resistance.

Keywords: *Klebsiella pneumoniae*, *luxS* gene, *acrA* gene, AgNPs, berberine

Резюме

Klebsiella pneumoniae е Грам-отрицателен опортюнистичен патоген и основна причина за инфекции, свързани със здравеопазването, в световен мащаб. Между септември и декември 2025 г. са събрани 200 клинични проби от пациенти от различна възраст и пол, посещаващи учебните болници Baquba и Al-Batool в Дияла, Ирак. Пробите са оцветени по Грам и култивирани върху кръвен агар, MacConkey агар и хромогенна среда за *K. pneumoniae*. От събраните проби 139 показват положителен бактериален растеж. Най-високата честота на изолатите на *K. pneumoniae* е открита в проби от урина (43.7%), докато пробите от храчки показват най-ниската честота (12.5%). Тестването за антимикробна чувствителност показва висока резистентност към амоксицилин-клавуланова киселина (93%), цефотаксим и доксициклин (87%) и триметоприм-сулфаметоксазол (81%), докато изолатите остават относително чувствителни към меропенем (75%) и имипенем (72%). Молекулярният анализ показва, че всички изолати (100%) носят гените *acrB* и *luxS*. Третирането със сребърни наночастици (AgNPs) значително намали експресията и на двата гена, с общи стойности на промяна от 0,45 за *acrB* и 0.33 за *luxS*, въпреки че един изолат (K1) показва леко увеличение на експресията на *acrB* (1,61 пъти). Третирането с берберин доведе до по-голямо намаляване на генната експресия, с общи стойности на промяна от 0.20 за *acrB* и 0.29 за *luxS* във всички изолати. Тези открития показват, че както AgNPs, така и берберинът потискат експресията на гени, свързани с антибиотична резистентност и кворум сетинг, в *K. pneumoniae*,

*Corresponding author: linaabdulameer@uodiyala.edu.iq
Acta Microbiol. Bulg. 2026; 42(03) <https://doi.org/10.59393/amb26420311>

като берберинът показва по-голяма инхибиторна активност. Следователно, тези съединения могат да служат като обещаващи адюванти за подобряване на антибиотичната ефикасност и намаляване на бактериалната резистентност.

Introduction

Klebsiella pneumoniae is a Gram-negative opportunistic pathogen and a major cause of healthcare-associated infections worldwide. It is commonly implicated in pneumonia, urinary tract infections, bloodstream infections, and wound or burn infections, particularly among hospitalized and immunocompromised patients (Jones *et al.*, 2024). In recent years, the rapid emergence of multidrug-resistant (MDR) *K. pneumoniae* strains has become a serious clinical challenge, limiting therapeutic options and increasing morbidity and mortality rates. The resistance of *K. pneumoniae* to antimicrobial agents is mediated by several mechanisms, including the production of extended-spectrum β -lactamases (ESBLs), carbapenemases, plasmid-mediated resistance genes, and active efflux systems (Veloo *et al.*, 2022). One of the most important efflux systems in Gram-negative bacteria is the AcrAB–TolC pump. The *acrB* gene encodes a key component of this system and contributes to the extrusion of multiple classes of antibiotics from the bacterial cell, thereby promoting multidrug resistance (Jang, 2023). Overexpression of *acrB* has been associated with decreased antibiotic susceptibility and treatment failure (Trampari *et al.*, 2023).

In addition to resistance mechanisms, virulence factors enhance the ability of *K. pneumoniae* to persist and cause infection. Quorum sensing is a regulatory system that enables bacteria to coordinate gene expression according to cell density (Karampatakis *et al.*, 2023). The *luxS* gene plays a central role in the production of autoinducer-2 (AI-2), a signaling molecule involved in intercellular communication (Guo *et al.*, 2026). Through quorum sensing, *K. pneumoniae* can regulate biofilm formation and other virulence-related traits. Biofilm development increases bacterial tolerance to antimicrobial agents and host defenses, making infections more difficult to treat (Meng *et al.*, 2022).

The growing challenge posed by MDR *K. pneumoniae* has stimulated interest in alternative or complementary antimicrobial strategies. Nanotechnology-based approaches have emerged as promising tools in this context. Silver nanoparticles (AgNPs) are widely studied for their broad-spectrum antimicrobial properties against Gram-positive and Gram-negative bacteria (Alaallah *et al.*, 2023). Their antimicrobial activity is attributed to multiple mechanisms, including disruption of cell

membrane integrity, generation of reactive oxygen species, interaction with cellular proteins, and interference with nucleic acids. Due to their multifactorial mode of action, AgNPs may reduce the likelihood of resistance development compared with conventional antibiotics (Shahzad, 2025). Various plant-derived compounds have also been explored for their potential as antimicrobial agents or adjuvants. Berberine, an isoquinoline alkaloid extracted from species of *Berberis* (family *Berberidaceae*), has been shown to possess antibacterial and antibiofilm effects, slow bacterial growth, and improve the efficacy of some antibiotics (Yang *et al.*, 2025). Several research studies report that berberine may regulate efflux pump systems and quorum sensing pathways, which modulate both resistance and virulence (Qassadi *et al.*, 2023; Ding *et al.*, 2025). However, its precise molecular effects on resistance- and quorum sensing-associated genes in clinical *K. pneumoniae* isolates are yet to be clarified.

Assessing these agents on major resistance- and virulence-associated genes is crucial to elucidate their possible therapeutic effect. Thus, this study was designed to evaluate the effect of AgNPs and berberine on *acrB* and *luxS* gene expression in clinical isolates of *K. pneumoniae*, as a way to explain their possible role in diminishing multidrug resistance and bacterial virulence.

Materials and Methods

Materials

Berberine hydrochloride was purchased from Nootropics Depot/ USA. Silver nitrate (AgNO_3) was purchased from BDH, Sigma-Aldrich.

Isolation and identification of *K. pneumoniae* isolates

A total of 200 clinical samples were collected from patients of various age groups and genders at Baquba Teaching Hospital and Al-Batool Teaching Hospital in Diyala, Iraq, between September and December 2025, under the supervision of a specialist physician. Gram staining of each sample was performed, and the samples were also inoculated on primary media (blood agar, MacConkey agar, *K. pneumoniae* chromogenic agar) (HiMedia Laboratories Pvt. Ltd., Maharashtra, India), using a sterile loop for pathogen isolation. Inoculated plates were incubated aerobically at 37°C for 24 hours. Bacterial identification was performed initially based

on colony morphology, Gram staining, and routine conventional biochemical tests, including catalase, oxidase, citrate, urease, indole, motility, and triple sugar iron (TSI) tests. The identification was subsequently confirmed using the automated VITEK® 2 Compact system (bioMérieux, Marcy-l'Étoile, France).

Antibiotic susceptibility testing

Antimicrobial susceptibility of the bacterial isolates was determined using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with CLSI (2023) guidelines. For inoculum preparation, three to five well-isolated colonies grown on nutrient agar were suspended in sterile normal saline and adjusted to the turbidity of the 0.5 McFarland standard (approximately 1.5×10^8 colony-forming units (CFU)/mL). The standardized suspension was uniformly inoculated onto Mueller–Hinton agar plates, followed by the application of antibiotic disks. After incubation, inhibition zone diameters were measured, and isolates were interpreted as susceptible, intermediate, or resistant based on CLSI criteria. The antibiotic panel comprised aztreonam, amoxicillin–clavulanic acid, ceftazidime, cefotaxime, imipenem, meropenem, amikacin, tobramycin, levofloxacin, doxycycline, and trimethoprim–sulfamethoxazole. Multidrug-resistant (MDR) *K. pneumoniae* isolates were defined as those exhibiting resistance to three or more antimicrobial classes (Magiorakos *et al.*, 2011).

Preparation of AgNPs solution

AgNPs solutions were prepared with deionized distilled water by Ultra sonic homogenizer at room temperature for 30 min in concentrations of (5200, 2600, 1300, 650, 325, 162.5, 81.25, 40.6, 20.3, 10.15, 5.07 µg/ml). The commercially obtained AgNPs had a nominal particle size of 15 nm and 99.5% purity (assay) according to the manufacturer's specifications (Heshmati *et al.*, 2015).

Minimum inhibitory concentration (MIC) of AgNPs against K. pneumoniae

The minimum inhibitory concentration (MIC) is the lowest concentration of AgNPs that inhibits visible bacterial growth. AgNPs solutions were prepared according to the manufacturer's instructions using Mueller–Hinton broth, with concentrations ranging from 5.07 to 5200 µg/mL in two-fold serial dilutions (5200, 2600, 1300, 650, 325, 162.5, 81.25, 40.6, 20.3, 10.15, 5.07 µg/mL). Tester strains were inoculated at a density of 1.0×10^8 CFU/mL. Following incubation at 37°C for 24 hours, the MIC for each strain was determined as the lowest con-

centration showing no visible growth. The MIC was defined as the lowest concentration of the tested agent that completely inhibited visible bacterial growth after incubation. The minimum bactericidal concentration (MBC) was determined by subculturing aliquots from tubes showing no visible growth onto Mueller–Hinton agar plates. The MBC was defined as the lowest concentration at which no bacterial colonies were observed after incubation, indicating complete bactericidal activity (Heshmati *et al.*, 2015).

Minimum inhibitory concentration (MIC) of berberine

A 1:2 serial dilution of Berberine HCl was made in Mueller–Hinton broth (MHB) in a 96-well round-bottom plate at concentrations of 500, 250, 125, 62.5, 31.25, 15.61, 7.81 and 3.91 µg/mL (CLSI, 2023). Bacterial inoculum was prepared from an MHB subculture of *K. pneumoniae*. The bacterial suspension was adjusted to 1.0×10^8 CFU/mL, corresponding to a 0.5 McFarland standard, and verified spectrophotometrically by measuring the optical density (OD) at 625 nm, which was maintained between 0.08 and 0.10 absorbance units (AU). Then a 1:200 dilution in MHB was made to yield a final concentration of 5.0×10^5 CFU/mL. In each well, the diluted bacterial solution (100 µL) was combined with 100 µL of Berberine serially diluted in individual wells, maintaining a final volume of 200 µL per well. Negative and positive growth controls were included by adding only MHB or *K. pneumoniae* in MHB, respectively. After incubation at 37°C for 24 hours, 20 µL of resazurin (0.015%) was added to each well, followed by an additional incubation of 2–4 hours to observe color changes. Wells in which the resazurin color remained unchanged (blue) were considered to exceed the MIC. The MIC was defined as the lowest concentration of Berberine at which no bacterial growth was observed.

*Detection of efflux pump *acrB* and *luxS* genes*

Genomic DNA was isolated from bacterial colonies using the G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Seongnam-si, Gyeonggi-do, South Korea) according to the manufacturer's instructions. Conventional PCR amplification was performed using an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at the primer-specific temperatures (56–58°C) shown in Table 1 for 45 s, extension at 72°C for 45 s, and a final extension at 72°C for 7 min. Real-time PCR (qPCR) was carried

Table 1. Primers' sequence of *acrB* and *luxS* genes

Primer	Oligo sequence (5'-3')	Product size bp	Annealing temp [°C]	Ref.
<i>acrB</i>	F-GCGTCATGGTAGTGGGTGAAG R- GCTTTTACCTGCACACCTGGT	105	58	Abid, 2023
<i>LuxS</i>	F- TGC GTGCCGAACAAAGAA R- CAGCCCATTTGGCGAGATA	139	56	Wang <i>et al.</i> , 2025
<i>rpoB</i> <i>housekeeping gene</i>	F-AGCGGTGCAGAATAAGTCACG R- GAAGCTGCTTTCCGTTCCGTA	140	58	Abid, 2023

out using an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at the corresponding primer-specific temperatures shown in Table 1 for 30 s, and extension at 72°C for 30 s.

Agarose gel electrophoresis of PCR products

The PCR amplification products were analyzed by electrophoresis on a 1.5% agarose gel prepared in 1× TBE buffer. Electrophoresis was carried out at 7 V/cm² for 90 min, and the amplified DNA fragments were visualized using a 100 bp DNA ladder as a molecular size marker.

Ethical approval

The samples were obtained according to Local Research Ethics Committee approval in the College of Science, University of Diyala, No. 25-ASJ-Sci 168 on 1/12/2025.

Results

Isolation of *K. pneumoniae*

The result of the present study demonstrated that 139 samples were positive for bacterial growth, while 29 samples were negative. The most positive isolates were from wound and urine samples. *K. pneumoniae* isolates were at the highest percent in urine samples (43.7%), then in wound samples (25%), while the lowest percent was in sputum samples (12.5%), as shown in Table 2.

Antimicrobial sensitivity test

K. pneumoniae showed high resistance to amoxicillin-clavulanic acid (93%), cefotaxime and doxycycline (87%), trimethoprim-sulfamethox-

azole (81%), and resistance to ceftazidime (62%), while it was sensitive to meropenem (75%) and imipenem (72%), as shown in Table 3.

The results in Table 4 showed that 59.4% (19 isolates) of *K. pneumoniae* were extensively drug resistant (XRD) while 40.6% (13 isolates) were multidrug resistant (MDR).

Minimum inhibitory concentration of berberine and AgNPs against *K. pneumoniae*

The MICs of berberine and AgNPs against five selected multidrug-resistant *K. pneumoniae* isolates were detected, which fell between 7.81 and 125 µg/mL for berberine and 40.6 to 162.5 µg/mL for AgNPs, as shown in Table 4.

Genotyping detection of efflux pump *acrB* gene

The result of Efflux Pump gene detection indicates that all studied isolates showed a positive result for the *acrB* gene (100%) with a band size (105 bp), as shown in Figure 1. Also, the *luxS* gene showed positive results in all *K. pneumoniae* studied isolates (100%) with a band size (139 bp), as shown in Figure 2.

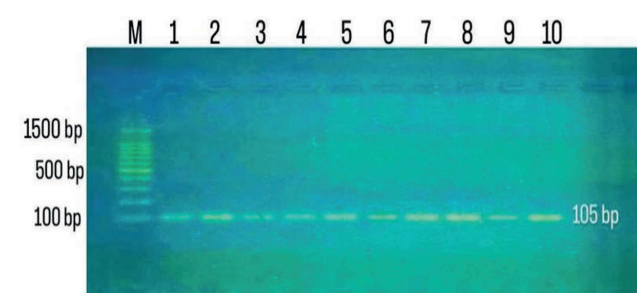


Fig. 1. PCR product for the *acrB* gene - the band size 105 bp. M - DNA ladder (100 bp).

Table 2. Results of bacterial isolates from clinical samples

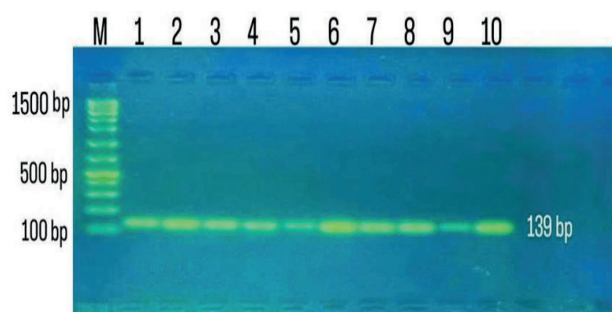
Clinical samples	Diagnosis of bacteria			Total
	No growth	Other species	<i>K. pneumoniae</i>	
Wound swabs	6 (14.7%)	37 (26.6%)	8 (25.0%)	50 (25.0%)
Burn swabs	11 (38.2%)	33 (23.7%)	6 (18.75%)	50 (25.0%)
Urine	5 (26.5%)	31 (22.3%)	14 (43.75%)	50 (25.0%)
Sputum	7 (20.6%)	37 (26.6 %)	4 (12.5%)	50 (25.0%)
Total	29 (100.0%)	139 (100.0%)	32 (100.0%)	200 (100.0%)

Table 3. Antibigram pattern of *K. pneumoniae* isolates(n=32)

Antibiotic	Resistance		Intermediate		Sensitive	
	Isolates No.	%	Isolates No.	%	Isolates No.	%
Aztreonam	15	46	5	16	12	38
Amoxicillin–clavulanic acid	30	93	-	-	2	7
Ceftazidime	20	62	-	-	12	38
Cefotaxame	28	87	-	-	4	13
Imipenem	7	22	2	7	23	72
Meropenem	5	16	3	9	24	75
Amikacin	19	59	4	13	9	28
Tobramycin	17	53	3	9	12	38
Levofloxacin	15	47	2	7	15	47
Doxycycline	28	87	1	3	3	9
Trimethopri-sulfamethoxazole	26	81	-	-	6	19

Table 4. MICs for berberine and AgNPs against *K. pneumoniae*

Isolates code	MIC of berberine (µg/ml)	MIC of AgNPs (µg/ml)
K1	62.5	81.25
K2	7.81	40.6
K3	125	162.5
K4	62.5	40.6
K5	7.81	81.25

**Fig. 2.** PCR product band size 139 bp for the *luxS* gene

Gene expression results of *acrB* before and after treatment with AgNPs and berberin

Table 5 shows the results of *acrB* gene expression in five *K. pneumoniae* isolates in three treatment statuses (treated with AgNPs, treated with berberin, and untreated isolates as a control) and using the *rpoB* gene as a housekeeping gene for internal comparison. The result showed that AgNPs-treated isolates showed a decrease in *acrB* gene expression, with fold values ranging from 0.04 to 0.32 in the four isolates (K2-K5), while the K1 isolate showed a slight increase (1.61) in gene expression. The overall gene folding was 0.45, indicating a decrease in gene expression in AgNPs-treated *K. pneumoniae* isolates compared with the untreated group.

The group of *K. pneumoniae* isolates treated with berberin showed a more inhibitory effect than AgNPs compared with untreated isolates because it showed a greater decrease in *acrB* gene expression in all isolates (K1-K5), with a range of fold values of 0.03-0.65, with a total overall folding 0.20.

Gene expression results of *luxS* before and after treatment with AgNPs and berberin

Table 6 shows the results for *luxS* gene expression in five *K. pneumoniae* isolates in three treatment statuses (treated with AgNPs, treated with berberin, and untreated isolates as a control) and using the *rpoB* gene as a housekeeping gene for internal comparison. The result showed that AgNPs-treated isolates showed a decrease in *LuxS* gene expression, with fold values ranging from 0.02 to 0.71 in all isolates (K1-K5). The overall fold value was 0.33, indicating a decrease in gene expression in AgNPs-treated *K. pneumoniae* isolates compared with the untreated group.

The group of *K. pneumoniae* isolates treated with berberin showed a more inhibitory effect than AgNPs compared with untreated isolates because it showed a greater decrease in *luxS* gene expression in all isolates (K1-K5), with a range of fold values from 0.14 to 0.53, with a total overall folding 0.29.

Table 5. Folding of gene expression of efflux pump gene (*acrB*) in *K. pneumoniae* isolates before and after treatment with AgNPs and berberin

Isolate code	State	Ct of H.K. (<i>rpoB</i>)	Ct of target <i>acrB</i>	ΔCt	$\Delta\Delta Ct$	(Folding)= $2^{-\Delta\Delta Ct}$	Avarage of folding
K1	Treated with AgNPs	18.65	24.89	6.24	-0.69	1.61	0.45
K2		21.80	28.60	6.80	1.65	0.32	
K3		22.70	27.36	4.66	2.14	0.23	
K4		19.90	23.60	3.70	4.30	0.05	
K5		17.00	27.84	10.84	4.70	0.04	
K1	Treated with berberin	17.48	25.04	7.56	0.63	0.65	0.20
K2		20.06	29.16	9.10	3.95	0.06	
K3		19.97	27.36	7.39	4.87	0.03	
K4		19.90	21.89	1.99	2.59	0.17	
K5		19.02	28.34	9.32	3.18	0.11	
K1	Untreated	16.97	23.90	6.93	0.00	1.00	1
K2		16.95	22.10	5.15	0.00	1.00	
K3		19.28	21.80	2.52	0.00	1.00	
K4		21.44	20.84	-0.60	0.00	1.00	
K5		18.06	24.20	6.14	0.00	1.00	

Table 6. Folding of gene expression of efflux pump gene (*luxS*) in *K. pneumoniae* isolates before and after treatment with AgNPs and berberin

Isolate code	State	Ct of H.K. (<i>rpoB</i>)	Ct of target <i>acrB</i>	ΔCt	$\Delta\Delta Ct$	(Folding)= $2^{-\Delta\Delta Ct}$	Avarage of folding
K1	Treated with AgNPs	18.65	24.40	5.75	5.32	0.02	0.33
K2		21.80	27.19	5.39	0.24	0.85	
K3		19.70	28.10	8.40	6.02	0.02	
K4		19.90	24.30	4.40	4.30	0.05	
K5		18.34	24.30	5.96	0.48	0.71	
K1	Treated with berberin	19.65	22.29	2.64	2.21	0.22	0.29
K2		19.82	26.43	6.61	1.46	0.36	
K3		20.37	23.66	3.29	0.91	0.53	
K4		18.99	21.88	2.89	2.79	0.14	
K5		20.22	28.24	8.02	2.54	0.17	
K1	Untreated	16.97	17.40	0.43	0.00	1.00	1
K2		16.95	22.10	5.15	0.00	1.00	
K3		19.28	21.66	2.38	0.00	1.00	
K4		21.44	21.54	0.10	0.00	1.00	
K5		18.06	23.54	5.48	0.00	1.00	

Discussion

K. pneumoniae isolates are emerging as community - and hospital-acquired infectious agents with various antibiotic resistance mechanisms. Antibiotic treatment poses a significant problem as *K. pneumoniae* isolates become fully resistant by producing ESBL and high-level AmpC beta-lactamase (Balıkcı *et al.*, 2014).

In the present study, *K. pneumoniae* showed

high resistance to amoxicillin-clavulanic acid (93%), cefotaxime and doxycycline (87%), trimethoprim-sulfamethoxazole (81%), and resistance to ceftazidime (62%), while it was sensitive to meropenem (75%) and imipenem (72%). This result agrees with (Aytaç, 2024), who showed that *K. pneumoniae* is highly resistant to amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole. Other studies have also recorded high sus-

ceptibility rates of 85.64% against meropenem and 63.61% for imipenem (Lagha *et al.*, 2021; Ngoi *et al.*, 2021). In a similar report, *K. pneumoniae* isolates showed high susceptibility to imipenem (Mapipa *et al.*, 2022). Low resistance rates of 35% and 34% to ertapenem and meropenem, which contradict our findings, have been reported elsewhere in South Africa (Ebomah and Okoh, 2020).

The results showed that (59.4%) of *K. pneumoniae* were XDR, while 40.6% were MDR. Another study recorded MDR (30.6%) and XDR (28.5%) *K. pneumoniae* (Dokmak *et al.*, 2025). Another study recorded that 82.5% were indicated as MDR *K. pneumoniae* (Odaa and Al-Mathkhury, 2025). The usage of antibiotics has been curtailed due to the emergence of multidrug-resistant bacterial diseases. There is a present necessity to enhance the efficacy of antibiotics. Currently, due to their cost-effectiveness and safety, the biosynthesis of nanoparticles (NPs) is being considered (Tahan *et al.*, 2024). In 2014, Chahardooli and Khodadadi (2014) conducted the biosynthesis of AgNPs using a plant extract and showed its notable antibacterial efficacy against nosocomial infections. The study by Mekky *et al.* (2021) evaluated the antibacterial efficacy of green-synthesized AgNPs against *K. pneumoniae*, *E. coli*, *Staphylococcus aureus*, and *S. haemolyticus*. The bactericidal activity of nanoparticles is largely dependent on both the dose and particle size. The antibacterial mechanism of AgNPs is multifactorial. Silver ions (Ag^+) exert antibacterial effects by interacting with the peptidoglycan layer of the bacterial cell wall, the thickness of which varies between Gram-negative and Gram-positive species, resulting in differential susceptibility (Ershov and Ershov, 2024). Additionally, the positive charge of AgNPs interacts with the negatively charged bacterial cell wall, leading to morphological changes, increased membrane permeability, and pore formation, ultimately causing bacterial death. Ag^+ can also bind to amino ($-\text{NH}_2$) and thiol ($-\text{SH}$) groups of membrane proteins, promoting reactive oxygen species (ROS) generation, inhibiting respiratory enzymes, and leading to cell death (Alaallah *et al.*, 2023). Other mechanisms include disruption of the cell wall, interaction with bacterial enzymes and DNA, ribosomal destabilization, and interference with the electron transport chain, collectively resulting in bacterial lethality (Menichetti *et al.*, 2023).

In recent decades, multidrug resistance (MDR) has become a growing concern due to the rise of efflux pumps such as *AcrAB* and *LuxS* in

MDR pathogens like *K. pneumoniae*. Higher levels of antibiotic resistance correlate with overexpression of *AcrAB* and *OqxAB* efflux systems. Here, we investigated the role of the *AcrAB* efflux pump on *K. pneumoniae* antibiotic resistance (Albarri *et al.*, 2024).

According to the study of Padilla *et al.* (2010), the *acrAB* gene promotes antibiotic resistance in bacteria by reducing the isolates' sensitivity to antimicrobial peptides and conventional antibiotics by drug expulsion from the cell. Additionally, Xu *et al.* (2019) found that clinical trials comparing antibiotic-resistant and susceptible *K. pneumoniae* isolates revealed greater levels of *acrB* expression, suggesting a direct relationship between excretory pumps and multidrug resistance patterns in this bacterium. In addition, it has been recently reviewed that resistant *K. pneumoniae* and *Escherichia coli* isolates often contain the *acrAB-tolC* gene. This gene is clearly linked to high resistance rates and various effective treatment lines, so future therapeutic strategies must target excretory pump mechanisms (Kharel *et al.*, 2025).

The results also showed that treatment with silver nano and berberine extract decreases the expression of *acrB* and *luxS* genes in *K. pneumoniae*. A recent study carried out by Navon-Venezia *et al.* (2017) found that modest concentrations of berberine (25 and 50 $\mu\text{g/ml}$) increased minimum inhibitory concentrations of efflux-related antibiotics, including rifampicin and azithromycin. Additionally, its low-dose use against Gram-negative bacteria was found to be dangerous. Many therapeutic plants contain berberine, a natural isoquinoline alkaloid with antibacterial and antifungal effects (Ghaima, 2022). When used with several antibiotics, berberine increases their ability to suppress microorganisms. A previous study found that berberine dose-dependently affects MRSA cell surface and changes saturated and unsaturated fatty acid levels, compromising membrane integrity (Ghaima, 2022).

A study by Foroohimanjili *et al.* (2020) in their study showed that AgNPs inhibit quorum sensing and virulence factors of *Klebsiella* by negatively regulating genes like *luxS*, resulting in diminished virulence and antibiotic resistance linked to the molecular mechanisms of this bacterium. There may be a lot of efflux inhibitors that operate at the protein level or by simply diffusing the ionic gradient needed to drive the antiport function, which makes gene expression research somewhat challenging. An inhibiting drug's effect on known ef-

flux pumps can be determined using real-time polymerase chain reaction (Siriyoung *et al.*, 2017). The study conducted by Ghaima (2022) shows that berberine has a bactericidal effect and so has potential application in clinical infections. This compound is of high importance in developing new antimicrobial agents against *K. pneumoniae*, especially for multidrug-resistant and/or hard-to-treat strains.

Conclusion

Our results demonstrated that both berberine and silver nanoparticles inhibited the expression of antibiotic resistance genes in *K. pneumoniae* isolates, with berberine demonstrating a relative advantage in this regard. These findings provide credence to the idea that these chemicals could be used as adjuvants to boost antibiotic effectiveness and decrease bacterial resistance. To fully assess their clinical efficiency and molecular mechanisms of action, additional research is needed.

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