DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

Effect of Sub-Inhibitory Concentrations of Ciprofloxacin, Curcumin, and Capsaicin on Quorum Sensing Gene Expression in Acinetobacter Baumannii Isolates

Yasameen Abdullah Ali [1], Kareem Ibrahim Mubarak [2]
[1,2]Department of Biology, College of Sciences, Diyala University, Diyala, 32001, Iraq.
Corresponding Author email:1 (Scibioms242513@uodiyala.edu.iq)

Abstract

Acinetobacter baumannii is a common Gram –negative, non-fermenting coccobacillus. Bacteria can live in blood, urine, skin, soft tissues, lungs, digestive tract, wounds, and brain. Form a biofilm and efflux pumps and is quite resistant to several drugs. The study proposed to consider the A. baumanni prevalence in different infections, to determine the MIC of ciprofloxacin, use molecular methods (PCR) to find genes, effect of ciprofloxacin, curcumin, and Capsaicin on Ggene expression of Quorum Sensing (QS). From October 8, 2025, to January 1, 2026, 160 l specimens were taken from different infections at Iraq's Baqubah Teaching Hospital.

The results showed that 20 of the isolates (12.5% of the total) had a positive growth rate for *A. baumannii*. The samples were sorted by where they came from: wounds, sputum, burns, and urine were 15%, 45%, 30%, 10% respectively. The study found the fold change values following treatment with ciprofloxacin, curcumin, and capsaicin, for the *abaI* gene were 0.7239, 0.0236, and 0.1453, respectively, while the change for *abaR* gene was 0.7100, 0.0122, and 0.3927, respectively. The effect of sub-MIC combination of ciprofloxacin with curcumin was more than with capsaicin, where the fold change values were recorded 0.0528 and 1.6353, respectively for *abaI,* while 0.0729 and 2.0507 for *abaR* gene.

The conclusion of this study is that the fold change value of quorum sensing *abaI*, *abaR* genes significantly changed, and after treatment with ciprofloxacin, curcumin, and capsaicin, in addition, the significant effect of ciprofloxacin and curcumin mixture gives promising ideas for suggesting alternative strategies to lowering the virulence factors controlled by QS, such as biofilm formation to resolve the antibiotics resistance problem for *A . Baumannii* isolates.

Keyword: Acinetobacter baumannii, Antibiotic, capsaicin, curcumin, Quorum Sensing genes.

1. Introduction

A. baumannii is a coccobacillus; Gam-negative that does not ferment lactose. It is common in nature and endemic in hospitals. This species is considered one of the most dangerous and prevalent MDR pathogens, which are the main reason for hospital-acquired infections in hospitals all over the world it is found worldwide. Found in medical equipment and surfaces, in addition, it possesses a high degree of environmental adaptability and drug resistance [1], [2]. It is one of the most powerful problematic multidrug-resistant bacteria and is responsible for about 500 deaths and 7300 infection

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

cases per year [3]. *A. baumannii* causes burn infection, when complications treatment becomes a difficult problem.

Other infections include septicemia when the bacteria reach the bloodstream and cause a systemic infection. Bacterial infections have also been connected to extended prolonged catheter use and antibiotic therapy [4]. Inside hospitals' critical care units, in hospital intensive care units, the mortality rate among patients with MDR infections was 26%; however, it increased to 43% in severe cases. In hospitals, the high rate of mortality among infections with multidrug resistance is up to (26 %) however, it increased to 43% [5]. The important pathogenesis factor found in bacteria is an outer membrane proteins (OMPs), which allow the bacteria to penetrate host epithelial cells and attach themselves to the host cells, releasing apoptotic factors in response to these proteins, resulting in cell death [6]. Capsular exopolysaccharides shield the pathogen from host-mediated and environmental stressors, and their makeup composition dictates the level of virulence [7].

A. baumannii isolates rapidly acquire resistance to antibiotics, that result in difficult of infections. This reflects the importance of investigating alternative antimicrobial targets for these bacterial isolates [8]. Furthermore, it can develop biofilms on non-living surfaces as well as living surfaces, which allows it to survive on surfaces that would normally be unsuitable for it, such as medical devices and hospital floors. It also has a wide variety of efflux pumps. The fact that antimicrobial medicine agents take longer to reach the biofilm and that those microorganisms grow more slowly inside the biofilm are two of the several major factors that make bacteria associated with biofilms more drug-resistant [9]. lipase enzyme, important virulence factors in bacteria that aid in the invasion and spread of bacteria, as well as the subsequent evolution of infection by breaking down the host's tissues [10].

Communication of one bacterium with another by quorum sensing (QS), through signalling molecules called autoinducers (AIs) that are located outside the cell. The release of bioluminescence, competence, spore formation, antibiotic synthesis, and formation of biofilm are all controlled by (QS) [11]. If this bacterial communication system is disrupted, it could reduce many virulence factors at once [12]. To inhibit QS, phytochemical compounds control how bacteria produce AHL. It is believed that the inhibition effects by these agents occur through either competing with or speeding up the breakdown of receptors of the LuxR/LasR that AHL molecules bind, or by preventing AHL activity altogether due to their similar structures [13]. Capsaicin is a bioactive alkaloid compound that is found in many species of peppers. It can stop the growth of microbes and has cardioprotective, antioxidant, neuroprotective, and cancer-fighting properties [14].

Our study aimed to isolate and identify *A. baumannii* from different infections, test whether or not these isolates could express efflux pumps, conduct a molecular study to find QS genes (*abaI*, *abaR*), to research the impact of ciprofloxacin, curcumin, and capsaicin on the gene expression of these genes when used as quorum sensing inhibitors either alone or in combination. Ciprofloxacin selection due to the absence of study for their effect in combination with curcumin and capsaicin on QS of *A. baummanii*, in addition to a previous study, revealed the higher synergistic effect of ciprofloxacin **w**ith curcumin and other bioactive compounds on another bacterial spp.

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

2. Materials And Methods

2.1 A. baumannii isolation and Identification

One hundred sixty pathogenic samples were collected from patients in Baquba Teaching Hospitals between October 8, 2025, and January 1, 2026. These specimens included sputum, urine, burn and wound swabs, and other clinical specimens. Infection location, age, sex, and patient name were documented. The samples were placed on MacConkey agar and incubated at optimum temperature (37°C0 for 24 hrs after inoculation. The growth colonies that were suspected to form were thereafter moved to chromium CHROM agar and left to incubate at 37°C for another 24 hours. Colony features and staining were used to identify distinct isolates. A. baumannii isolates were identified using a battery of biochemical tests, including the IMViC tests, catalase, and oxidase test [15]. For the purpose of very accurate bacterial identification, the automated VITEK-2 system was employed. Afterwards, the Gram-negative VITEK-2 ID-GN card and the bacterial tubes were carefully inserted into the VITEK-2 apparatus. The program was operated in accordance with the guidelines given by Biomerieux, a French firm [16].

2.2 Minimum Inhibitory Concentration (MIC)

A microtiter plate method was used for the determination of the MIC of ciprofloxacin, curcumin, and capsaicin. Antimicrobial agents were diluted in Mueller Hinton Broth to prepare the following concentrations: 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 µg /mL. Then, add 50 µL to each well except for the negative control wells, which were inoculated with 50 µL of a bacterial suspension. For the past 18–24 hours, microtiter plates have been placed in optimum temperature for growth (37°C). The colour of the resazurin broth test was altered from blue to pink using the lowest amount of dye, 20 µL, which was added to each well, followed by further incubation for 2 to 4 hours [17].

2.3 Efflux Pump investigation

Determining the efflux pumps action for isolates (Cartwheel technique), Ethidium Bromide agar was used. The following test was conducted in order to find out whether Efflux pumps were present [18], 5 milliliters of an appropriate liquid broth were used to cultivate the bacterial isolates overnight at 37ºC. Concentrations of 0.5, 1, 1.5,2, and 2.5 µg/mL from EtBr in Tryptic soy agar plates protected from light and streaked from bacterial suspension. Dry plates at room temperature, then place them in the incubator at 37°C overnight. The results were then examined under ultraviolet light with a UV transilluminator.

2.4 Molecular Study

2.4.1 Gene detection by polymerase chain reaction (PCR)

Molecular study was the detection of three genes detection including the A. baumanii identification gene (blaOXA-51), two QS genes abaI and abaR, in ten Isolates with the following steps:

A genomic DNA purification kit (USA, Promega) was used for genomic DNA extract action, we isolated genomic DNA from *A. baumannii* isolates. We then used a NanoDrop spectrophotometer to check the DNA purity. Following this, the genomic DNA was quantified and then preserved at -20°C. Oligonucleotide sequences for primers (Table 1), primer amplification protocol: The PCR mixture's ultimate volume was 25 µL: 12.5 µL of Master Mix 2x, 1 µL of each of forward and reverse primers, 5 µL of template DNA, and 5.5 µL of nuclease-free water. This was done in uniplex

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

PCR Eppendorf tubes, but the amount changed in multiplex PCR. The mixture was briefly mixed using a vortex and then placed in a thermocycler. The polymerase chain reaction used in this study initial denaturation at 95°C for 4 minutes, No. of cycle 35, denaturation at 94°C/ 60 seconds, annealing at 52°C for 30 seconds for *abaI* and *abaR* genes, 53°C/30 sec for bla_{OXA-51} gene, elongation at 72°C for 90 seconds for *abaI* and *abaR* genes, 72°C for 60 seconds for bla_{OXA-51} gene, and final extension at 72°C for 5 minutes.

Primer dilution: The oligonucleotide primers, initially in lyophilized form, were dissolved and diluted in nuclease-free distilled deionized water DDW according to the manufacturer's instructions to achieve a concentration of 100 picomol/µL. Subsequently, this stock solution was further diluted in nuclease-free distilled deionized water DDW. To reach approximately 10 picomol/µL. This technique was applied to all primers in this study, as listed in Table 1. To identify PCR products that had been amplified, we employed gel electrophoresis. They were documented using a UV transilluminator and ethidium bromide dye for visualization. A 100 mL solution of 1x TBE buffer containing 1 gram of agarose was prepared. The gel was submerged with TBE buffer, the tank was sealed, and the DNA extract and PCR products were electrophoresed for one hour at 5 volts/cm2 of the gel. Once electrophoresis was complete, the agarose gel was taken out of the tank, photographed, and analyzed visually using a UV transilluminator documentation system [19]. According to [20] 5µL singleplex PCR products were added to each PCR reaction well. Each electrophoresis run was accompanied by the use of a DNA ladder to verify the size of the PCR products. It was made feasible to observe DNA bands via the UV transilluminator documentation system.

Table 1: Oligonucleotide Primer Sequences (Alpha DNA Company, USA)

Primer Name	Sequence 5`-3`	Product size (bp)	Reference
abaI	F- AAAGTTACCGCTACAGGG R- CACGATGGGCACGAAA	435	[21]
abaR	F- TCCTCGGGTCCCAATA R- TAAATCTACCGCATCAA	310	[21]
bla_{OXA-51}	F- TAA TGC TTT GAT CGG CCT TG R- TGG ATT GCA CTT CAT CTT GG	353	[22]
16 S rRNA H. k gene	F- TCC TAC GGG AGG CAG CAG T R- GGA CTA CCA GGG TAT CTA ATC CTG TT	448	[23]

H. K: housekeeping

2.4.2 Gene Expression study by RT-PCR reaction of abaI and abaR genes

The quantitative RT-PCR reaction was performed utilizing two resistant isolates from sputum and burn swab specimens. The isolates were exposed to five treatments, including sub-MIC of capsaicin, curcumin, ciprofloxacin, and a combination of both agents to detect the impact of antibiotics (Ciprofloxacin) and natural substances, curcumin and capsaicin, on the fold value of the quorum sensing gene (abaI, abaR). To extract and purify the RNA, we employed the TRIzol Reagent technique. A Quantus Fluorometer (Promega, USA) was employed to determine the concentration of RNA in the sample in accordance with the supplier's instructions and protocols. In order to conduct real-time PCR, the Gene 9600 Quantitative Instrument was utilized.

Primers were designed to be compatible with the abaI A and abaR genes. We determined the Cycle Threshold (CT) value by conducting a thermal reaction with the solution we produced using a Real-Time PCR Cycler. During the amplification process in the course of the heat reaction, the

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

computer records the CT values that were obtained [24]. The following measurements used the ΔΔCT method, as described in reference [25], to determine variations in gene expression levels.

ΔCT = CT target gene - CT housekeeping gene (*16SrRNA*)

ΔΔCT = ΔCT Treated - ΔCT Control

Folding = $2^{-\Delta\Delta CT}$

2.5 Statistical analysis

To analyse how various groups or variables affected the study's data, we used the Statistical Package for the Social Sciences SPSS [26].

3. Results and Discussion

3.1 Identification of A baumanni isolates

The results after incubation overnight at 37° C show that 80 out of 160 (or 50%) of the positive growth was inoculated as primary on MacConkey agar and CHROM agar medium. The identification was then validated using standard procedures. The percentage in Fig. 1 showed A. baumannii isolates formed 20 (12.5%), whereas 60 /160 showed other species of bacteria according to colony features, which appeared small, pale, pink, and did not ferment lactose when isolates were grown on MacConkey agar. While colonies cultivated on CHROM agar medium, they revealed a light purple colour and had a halo surrounding them. Gram staining revealed red Gram -negative coccobacilli that occasionally appeared in pairs or short chains. Biochemical tests of isolates were negative for oxidase, the production of urease and indole, methyl red, Voges – Proskauer tests, whereas they are positive for catalase, and citrate utilization test. Kligler iron agar showed an alkaline slant. With no change in the bottom, without gas production, H2S was negative [15]. Fifty specimens showed no growth; this result is similar to [27], [28], who recorded the percentage of bacteria (12 %) and (13.31%), respectively. Our finding is higher than the finding of [29] were they recorded (9.7 %).

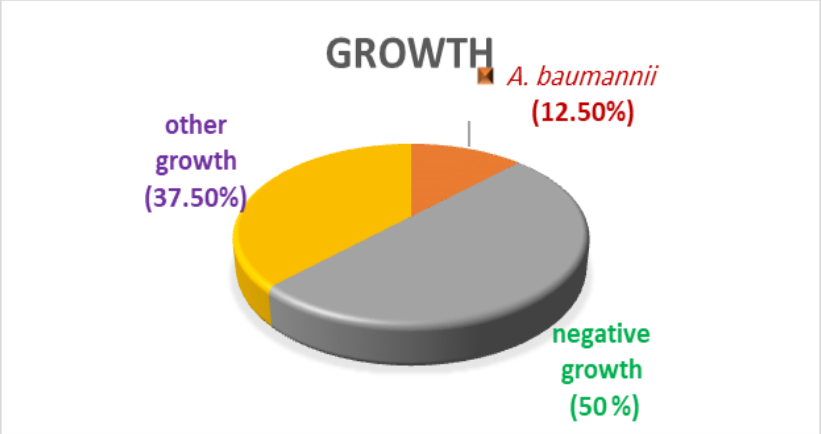

Fig. 1 The percentages of bacteriological growth for 160 specimens

The results in Table 2 represent the prevalence of (20) *A. baumannii* isolates based on the source of infection and sex, which were in sputum 9 (45%), burns 6 (30%), wounds 3 (15 %) and urine 2 (10 %). The prevalence of isolates among males and females was 7 (35) and 13 (65), respectively. These results were corresponding to [30], he found that the distribution of *A. baumannii* isolates was

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

in burn (50%), wound (13.45%) Urine (10%). At the same time, the finding nearly to [31] they recorded the percentage of bacterial isolates was from sputum 17 (42.5%), urine 7 (17.5%), and wounds 10 (25%).

Table 2: A. baumannii isolates distribution based on the source and sex.

(20) specimens	Percentage of positive *A.b* of Male	Percentage of positive *A.b* of Female	Percentage of positive *A.b* of Total
	No (%)	No (%)	No (%)
Sputum	3 (15)	6 (30)	9(45)
Burns	2 (10)	4 (20)	6(30)
Wounds	1 (5)	2 (10)	3(15)
Urine	1 (5)	1 (5)	2(10)
Total	7 (35)	13 (65)	20(100)

A.b: *Acinetobacter baumannii*

3.1.1 Hemolysis Production Test

It was used to investigate the production of the hemolysis enzyme. In the current study, all 20 isolates were streaked on blood agar and incubated under optimum conditions at 37Cin whole day on blood agar. The colonies of *A. baumannii* isolates looked white, smooth, round, and cream-colored with whole edges. All 20 (100%) isolates showed negative hemolysis. Colonies became more mucoidal during prolonged incubation. This finding was consistent with numerous previous studies, including [27].

3.2 Minimum Inhibitory Concentration (MIC) of Ciprofloxacin

The ciprofloxacin MIC for each of the twenty isolates was estimated using the broth microdilution standards method. Ciprofloxacin (MIC) values ranged between 32 and 1024 μg/mL, depending on the isolates, as shown in Table 3.

Table 3: Ciprofloxacin MIC (ug /ml) for (20) A. baumannii isolates (Breakpoint ≤1(S)/ ≥ 4(R).

Isolates series	MIC (ug/ml)	Isolates series	MIC (ug/ml)
A. b 1	256	A. b 11	128
A. b 2	1024	A. b 12	64
A. b 3	512	A. b 13	256
A. b 4	512	A. b 14	512
A. b 5	128	A. b 15	512
A. b 6	64	A. b 16	256
A. b 7	64	A. b 17	32
A. b 8	256	A. b 18	256
A. b 9	32	A. b 19	128
A. b 10	32	A. b 20	256

A.b*: Acinetobacter baumannii,* MIC: Minimum Inhibitory Concentration

3.3 Efflux pump production

Efflux pump activity was detected in all isolates, with varying intensities ranging from low to high among different isolates. The finding of efflux pump activity revealed that 14 (70%) of the isolates glowed under UV light in the presence of ethidium bromide. indicating efflux pump activity ranging from low to moderate levels. On the other hand, 6 (30%) of the isolates, including isolates 2, 5, 6, 11, 14, and 20, remained non-fluorescent even at the highest tested concentration (2.5 mg/L), suggesting high efflux pump activity. This finding suggests that they have strong efflux pump activity that can

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

quickly remove ethidium bromide from the bacterial cells. These results are consistent with earlier research showing that clinical isolates frequently include efflux pumps. The phenotypic analysis using the cartwheel method using Ethidium Bromide (EtBr) agar revealed that out of the fifteen strains tested, only nine (60%) produced efflux pumps [32]. There was another investigation that discovered it in 68% of the isolates [33].

3.4 Molecular Study

3.5.1 Detection of Target genes by Polymerase Chain Reaction (PCR)

The blaOXA-51 gene was detected to confirm the diagnosis, and two QS genes of ten clinical isolates of *A. baumannii* were successfully detected using the molecular technique, the polymerase chain reaction (PCR). The polymerase chain reaction (PCR) is a widely used and efficient method for identifying bacterial species and specific genes [34].

3.5.1.1 Molecular Detection of the blaOXA-51 gene

The blaOXA-51 gene was found in all ten isolates (100 %) in this study. Fig. 2 shows DNA bands with a size of 353 bp. Previous studies have shown that the blaOXA-51 gene is present in all A. baumannii clinical isolates in Iraq (100%), lending credence to its use as a molecular marker for species identification [35], [30]. Similarly, [36] found that the blaOXA-51 gene was present in all A. baumannii clinical isolates (100%).

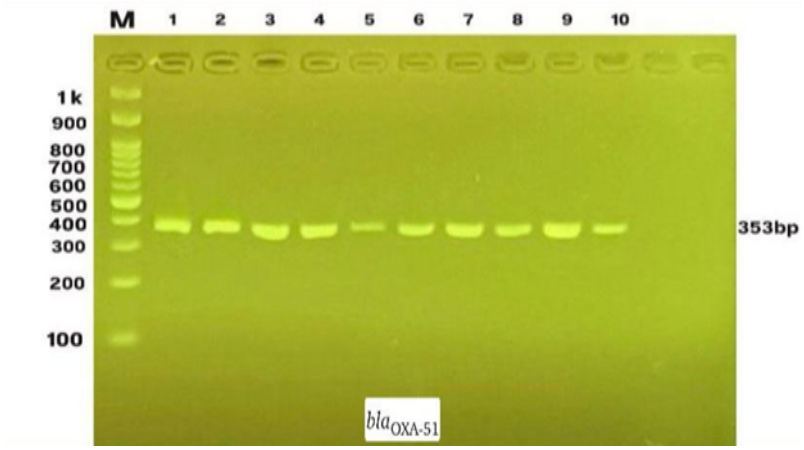

Fig. 2 Agarose gel electrophoresis (1.5% agarose,7v/cm2 for 60 min) for blaOXA-51 gene 353 bp amplicon) lane 100 bp DNA Ladder.

3.5.1.2 Molecular Detection of quorum sensing abaI Gene

The gene identification results from this study indicated that 7 (70%) isolates carried the abaI gene. displayed the DNA bands with a size of 435 bp in Fig. 3. The abaI were widely distributed among A baumannii isolates in several studies. For example, [37] reported 78.75% prevalence of abaI, while another study reported the appearance of the abaI gene in 16 (88.8%) [38]. whereas the other study found that 8 (61.54%) of the isolates had the abaI gene, indicating its role in quorum sensing [39]. The abaI gene is a gene that controls quorum sensing and makes an autoinducer synthase. The isolates have the ability to produce QS signalling molecules that contain abaI, and the changes in the abaI gene affected the ability of Acinetobacter spp. to form biofilms [40].

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

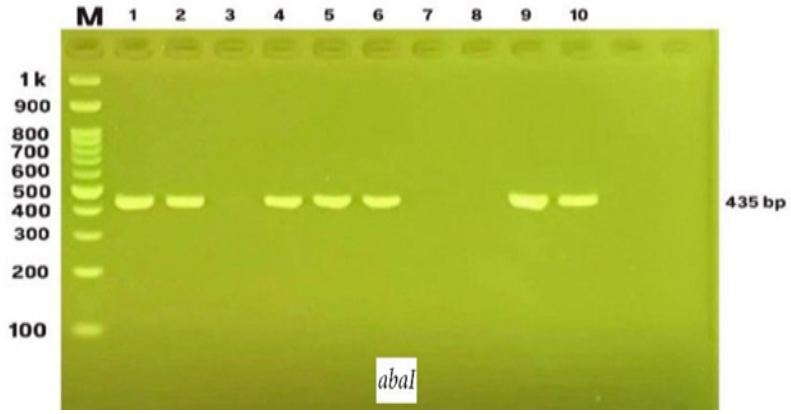

Fig. 3 Agarose gel electrophoresis (1.5% agarose, 7V/cm2 for 60 min) for abaI gene (435 bp amplicon), lane 100 bp DNA Ladder.

3.5.1.3 Molecular Detection of Quorum Sensing (abaR) gene

The results of the gene detection in this study revealed that 7 (70%) isolates carried the abaR gene. Fig. 4 displayed the DNA bands with a size of 310 bp. The quorum-sensing genes abaI and abaR were widely distributed among A. baumannii isolates in several studies. A previous study revealed that the prevalence of abaI 78.75% [37]. whereas another study showed the presence of 10 (55.5%) abaR gene in 18 isolates of A. baumannii [38].

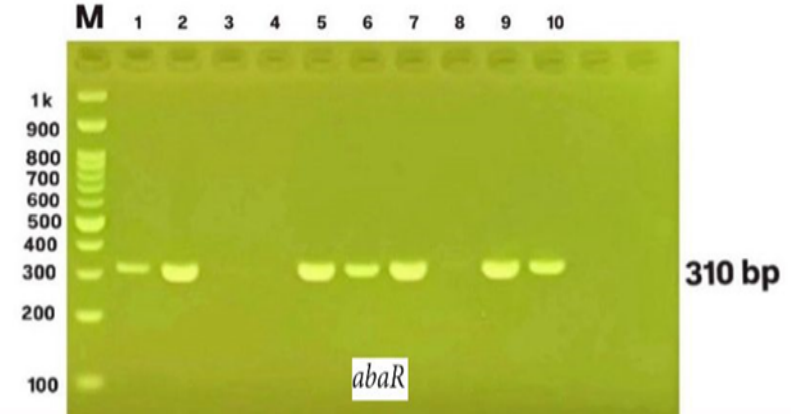

Fig. 4 Agarose gel electrophoresis (1.5% agarose, 7V/cm2 for 60 min) for abaR gene (310 bp amplicon), lane 100 bp DNA Ladder.

3.5.2 Gene expression study by qRT -PCR technique

The current study depended on qRT-PCR) Technique employing two resistant isolates of A. baumannii obtained from sputum and burn swab samples. The isolates were subjected to five different treatments to see how antibiotics and natural substances affected the fold value of genes under study. The treatments for each isolate included sub-MIC ciprofloxacin (16 ug/ml), sub-MIC curcumin (64 ug/ml), and a combination of ciprofloxacin and curcumin at a sub-MIC of each isolate. Sub-MIC concentration of capsaicin (64 ug/ml) for one isolate, (128 ug/ml) for another isolate, and a mix of ciprofloxacin and capsaicin (sub-MIC for each isolate).

By comparing the target and reference Ct values (including the housekeeping gene and quorum sensing genes, the results of the qRT-PCR tests confirmed genes expression levels were evaluated using the following equations, which were based on the relative quantification of gene expression

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

levels using the Livak formula ($2^{\wedge-\Delta\Delta Ct}$) method, as described in the materials and methods section [25]

3.5.2.1 Effect of Ciprofloxacin and Curcumin on abaI Gene Expression

We performed RT-PCR to evaluate the change in fold value of quorum-sensing genes as a result of treatment. This investigation showed that after growth in sub-MIC ciprofloxacin, curcumin, and a mix of both agents, the expression of abaI decreased in all isolates. The folding change value was recorded for ciprofloxacin, curcumin, and their combination was 0.7239, 0.0236, and 0.0528, respectively. A significant difference was observed, p = 0.0267, demonstrating that the combination of ciprofloxacin and curcumin was markedly more efficient in reducing abaI expression than either treatment individually Table 4.

Similar finding were reported in agreement with our results by the finding of [41] who demonstrated that they explained that curcumin alone exhibited lower antibacterial activity has low effect than its mixture with colistin in A baumanni isolates, another study reported that this antibiotic damages the outer membrane of bacteria, and demonstrated that this damage allows curcumin to easily penetrate the cell through the membrane and enhanced to antibacterial effect These mechanisms enhance the efficacy of antibiotics in eradicating bacteria, potentially yielding a synergistic effect superior to the antibiotics used in alone [42].

Curcumin is a plant polyphenolic active compound that has anti-inflammatory, antiproliferative, antibacterial, antibiofilm, and quorum-sensing inhibitory properties [43]. Several studies have suggested that it may act synergistically **w**ith other antibiotics against both classes of bacteria (Gram-positive and negative) [44], [43, [45]. Studies have shown that this compound shows interference with the cytoplasmic membrane, can easily permeate lipid bilayer due to its lipophilic nature, this result increase the permeability, in addition to causing a change in structural integrity, resulting in bacterial cell death [46].

Table 4: Effect of Cip and Cur treatments on the folding value of the abaI gene

Isolate	*16srRNA*	*AbaI*	ΔCT	ΔΔCT	Folding	Mean	Folding change
A.b 9	15.86	18.82	2.96	0.46	0.727	1.051	1.000
A.b 10	16.61	18.65	2.04	-0.46	1.376		
A.b 9 (CIP)	19.17	21.69	2.52	0.02	0.9862	0.7611	0.7239
A.b 10 (CIP)	17.60	21.00	3.40	0.90	0.5359		
A.b 9 (Cur)	14.52	23.38	8.86	6.36	0.012	0.025	0.0236
A.b 10 (Cur)	15.10	22.34	7.24	4.74	0.037		
A.b 9 (Cip + Cur)	15.76	22.15	6.39	3.89	0.0675	0.0555	0.0528
A.b 10 (Cip+Cur)	15.34	22.36	7.02	4.52	0.0436		
(P-value ≤0.01)*	(0.0267)*						

A.b =*Acinetobacter baumannii*, CIP=Ciprofloxacin, Cur =Curcumin

3.5.2.2 Effect of Ciprofloxacin and Capsaicin on abaI Gene Expression

When comparing ***abaI*** gene expression among resistant isolates that had been treated with sub-MIC levels of ciprofloxacin and capsaicin and a mix of both agents, this finding revealed a significant difference (p = 0.0087** when fold-change values were recorded as 0.7239, 0.1453, and 1.6353 (Table 5). The *Capsicum* genus is considered the plant source of capsaicin as a bioactive phytochemical [47]. In addition to discovering natural alternatives to antibiotics, this is crucial for patients for whom certain antibiotics may be toxic or cause side effects, such as allergies [48] and liver toxicity [49].

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

According to a study by [50], capsaicin revealed antibacterial action against *A. baumannii* at a 64 µg/mL concentration. The compound causes inhibition of the production of violacein in a bout fifty percentage from *C. violaceum*. Capsaicin, curcumin, and resveratrol were discovered to have an inhibitory effect on violacein synthesis 50% in the model organism *Chromobacterium violaceum*, which has been used in quorum sensing investigations. This suggests that these compounds interfere with pathways involved in quorum sensing. [51], [52] The anti-quorum-sensing activity may result from the combined effects of phytochemicals on auto-inducer signalling molecules, which control QS activities. This activity has been observed in plant extract. Some phytochemicals inhibit bacterial AHL production, thereby suppressing quorum sensing [13].

Table 5: Effect of Cip and Cap treatments on the folding value of abaI gene

Isolate	*16srRNA*	*AbaI*	ΔCT	ΔΔCT	Folding	Folding Mean	Folding change
A.b 9	15.86	18.82	2.96	0.46	0.727	1.051	1.00
A.b 10	16.61	18.65	2.04	-0.46	1.376		
A.b 9 (CIP)	19.17	21.69	2.52	0.02	0.9862	0.7611	0.7239
A.b 10 (CIP)	17.60	21.00	3.40	0.90	0.5359		
A.b 9 (Cap)	17.51	21.72	4.21	1.71	0.306	0.153	0.1453
A.b 10 (Cap)	18.22	41.95	23.73	21.23	0.0000004		
A.b 9 (Cip + Cap)	13.69	21.36	7.67	5.17	0.028	1.719	1.6353
A.b 10 (Cip+Cap)	22.20	22.93	0.73	-1.77	3.411		
P-value = (P≤0.01). **	0.0087**						

A.b =*A. baumannii*, CIP=ciprofloxacin, Cap =capsaicin.

3.5.2.3 Effect of Ciprofloxacin and Curcumin on abaR Gene Expression

We compared the fold value of the quorum sensing *abaR* gene in each treatment; Table 6 showed that following treatment with sub-MIC levels of ciprofloxacin and curcumin, and their sub-MIC combination. The corresponding fold-change values for ciprofloxacin, curcumin, and their combination were 0.7100, 0.0122, and 0.0729, respectively. The results revealed a highly different fold change value ($p = 0.0287$), indicating a clear decrease in *abaR* gene expression following treatment.

Curcumin inhibits the DNA-binding activity of LuxR-type regulators in *Vibrio harveyi*, curcumin modifies QS gene expression, and prevents the appearance of QS-controlled characteristics [53]. Treatments are to use natural quorum sensing inhibitors (QSIs) along with regular antibiotics treatments is to use natural quorum sensing inhibitors (QSIs) along with regular antibiotics. This interference reduces bacterial resistance to antibiotics. Natural QSIs like eugenol (from clove), curcumin (from turmeric), quercetin (from fruits), and furanones (from marine algae) have been shown to work when combined with antibiotics such as β-lactams, aminoglycosides, and fluoroquinolones [54]. As a result of this interference, bacteria become less capable of resisting antibiotic numerous researches recorded natural inhibitors of QS derived from various plant sources, such as curcumin (turmeric), furanones (marine algae), quercetin (fruits), and eugenol (clove) complement antibiotic activity [54].

Previous studies [55, 56] revealed that curcumin inhibits virulence factors regulated by QS whether used alone or in combination with antibiotics like azithromycin and gentamicin. The most notable effect of curcumin on QS-regulated virulence factors was pyoverdine synthesis, a siderophore essential for the survival and pathogenicity of *P. aeruginosa* during infection [57, 58], as

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

demonstrated in previous studies. Due to its ability to inhibit both QS-regulated virulence proteins and the type III secretion system (T3SS), curcumin represents a promising candidate for broad-spectrum anti-virulence therapy [58]. Curcumin has been shown to inhibit quorum sensing and violacein production in *Chromobacterium violaceum* by up to (50%) in Concentrations as low as 6 μM are sufficient to achieve this effect [51].

Table 6: Effect of Cip and Cur treatments on the folding value of abaR gene

Isolate	*16srRNA*	*AbaR*	ΔCT	ΔΔCT	Folding	Mean	Folding change
A.b 9	15.86	19.94	4.08	0.58	0.671	1.080	1.00
A.b 10	16.61	19.54	2.93	-0.58	1.490		
A.b 9 (CIP)	19.17	22.68	3.51	0.00	0.9965	0.7671	0.7100
A.b 10 (CIP)	17.60	22.00	4.40	0.89	0.5377		
A.b 9 (Cur)	14.52	24.88	10.36	6.85	0.009	0.013	0.0122
A.b 10 (Cur)	15.10	24.42	9.32	5.82	0.018		
A.b 9 (Cip + Cur)	15.76	22.95	7.19	3.68	0.0778	0.0788	0.0729
A.b 10 (Cip+Cur)	15.34	22.49	7.19	3.64	0.0799		
(P-value (P≤0.01) *.	0.0287*						

A.b =*A. baumannii*, CIP= Ciprofloxacin, Cur = curcumin.

3.5.2.4 Effect of Ciprofloxacin and Capsaicin on abaR Gene Expression

The results of *abaR* gene presented in Table 7 revealed significant differences, p = 0.0071, in the folding changes value of two resistant isolates before and after treatment with sub-MIC levels of ciprofloxacin and capsaicin, and their combined sub-MIC treatment, recorded as 0.7100, 0.3927, and 2.0507. These findings indicate significant alterations in *abaR* gene expression following treatment, as presented in Table 7. A study conducted by [54] reported that some QSIs directly block transcriptional regulators, thereby suppressing QS-dependent genes involved in virulence and antibiotic resistance. To inhibit QS, phytochemical compounds control how bacteria produce AHL. It is believed that the inhibition effects by these agents occur through either competing with or speeding up the breakdown of receptors of the LuxR/LasR that AHL molecules bind, or by preventing AHL activity altogether due to their similar structures [13]. Polyphenols luteolin and capsaicin are present in a wide variety of peppers, but mostly those belonging to the genus Capsicum. In addition to preventing microbial development, they have anti-inflammatory, antioxidant, cardio-protective, neuro-protective, and anticancer properties [14].

Table 7: Effect of Cip and Cap treatments on the folding value of the abaR gene

Isolate	*16srRNA*	*AbaR*	ΔCT	ΔΔCT	Folding	Folding Mean	Folding change
A.b 9	15.86	19.94	4.08	0.58	0.671	1.080	1.00
A.b 10	16.61	19.54	2.93	-0.58	1.490		
A.b 9 (CIP)	19.17	22.68	3.51	0.00	0.9965	0.7671	0.7100
A.b 10 (CIP)	17.60	22.00	4.40	0.89	0.5377		
A.b 9 (Cap)	17.51	22.85	5.34	1.83	0.280	0.424	0.3927
A.b 10 (Cap)	18.22	22.54	4.32	0.81	0.5684085		
A.b 9 (Cip + Cap)	13.69	24.04	10.35	6.84	0.009	2.216	2.0507
A.b 10 (Cip+Cap)	22.20	23.56	1.36	-2.15	4.423		
(P-value = (P≤0.01) **	0.0071**						

A.b = *A. baumannii*, CIP=Ciprofloxacin, Cap =Capsaicin

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

4. Conclusions

A. baumannii is a major clinical pathogen characterized by remarkable antimicrobial resistance and virulence traits, in present study according to this study. Twenty *A. baumanii* isolates 12.5% were identified, all isolates (100 %) carried the bla_{OXA-51} gene, and while 70% of the isolates possessed the quorum sensing genes *abaI* and *abaR*. the inhibitory effect of the natural compounds on the QS system this could play a principal role in treatment multi drugs resistant isolates. The findings regarding the synergistic effect of natural compounds with antibiotics are promising, sub-minimum inhibitory concentration (sub-MICs) of ciprofloxacin and curcumin because decreased quorum sensing gene expression. Tackling quorum sensing could complement conventional antibiotics, according to these findings this has the potential to lessen the intensity and treatment difficulty associated with of *A. baumannii* infections.

Conflict of Interest: There are no conflicts of interest regarding to this study

References

[1] Al-Shamiri, M. M., Zhang, S., Mi, P., Liu, Y., Xun, M., Yang, E., Ai, L., Han, L., & Chen, Y. (2021). Phenotypic and genotypic characteristics of Acinetobacter baumannii enrolled in the relationship among antibiotic resistance, biofilm formation and motility. Microbial Pathogenesis, 155, 104922. https://doi.org/10.1016/j.micpath.2021.104922

[2] Wang, X., & Qin, L.-J. (2019). A review on Acinetobacter baumannii. Journal of Acute Disease, 8(1), 16–20. https://doi.org/10.4103/2221-6189.250373

[3] Ahuatzin-Flores, O. E., Torres, E., & Chávez-Bravo, E. (2024). Acinetobacter baumannii, a multidrug-resistant opportunistic pathogen in new habitats: A systematic review. Microorganisms, 12(4), 644. https://doi.org/10.3390/microorganisms12040644

[4] Castanheira, M., Mendes, R. E., & Gales, A. C. (2023). Global epidemiology and mechanisms of resistance of Acinetobacter baumannii-calcoaceticus complex. Clinical Infectious Diseases, 76(Suppl 2), S166–S178. https://doi.org/10.1093/cid/ciad109

[5] Greene, C., Vadlamudi, G., Newton, D., Foxman, B., & Xi, C. (2016). The influence of biofilm formation and multidrug resistance on environmental survival of clinical and environmental isolates of Acinetobacter baumannii. American Journal of Infection Control, 44(5), e65–e71. https://doi.org/10.1016/j.ajic.2015.12.012

[6] Zhang, W., Zhou, H., Jiang, Y., He, J., Yao, Y., Wang, J., Liu, X., Leptihn, S., Hua, X., & Yu, Y. (2022). Acinetobacter baumannii outer membrane protein A induces pulmonary epithelial barrier dysfunction and bacterial translocation through the TLR2/IQGAP1 axis. Frontiers in Immunology, 13, 927955. https://doi.org/10.3389/fimmu.2022.927955

[7] Talyansky, Y., Nielsen, T. B., Yan, J., Carlino-Macdonald, U., Di Venanzio, G., Chakravorty, S., et al. (2021). Capsule carbohydrate structure determines virulence in Acinetobacter baumannii. PLoS Pathogens, 17(2), e1009291. https://doi.org/10.1371/journal.ppat.1009291

[8] Green, E. R., Juttukonda, L. J., & Skaar, E. P. (2020). The manganese-responsive transcriptional regulator MumR protects Acinetobacter baumannii from oxidative stress. Infection and Immunity, 88(3), e00762-19. https://doi.org/10.1128/IAI.00762-19

[9] Saipriya, K., Swathi, C. H., Ratnakar, K. S., & Sritharan, V. (2020). Quorum-sensing system in Acinetobacter baumannii: A potential target for new drug development. Journal of Applied Microbiology, 128(1), 15–27. https://doi.org/10.1111/jam.14330

[10] Pereira, D. G., Batista, E., de Cristo, T. G., Santiani, F., Sfaciotte, R. A. P., Ferraz, S. M., de Moraes, A. N., & Casagrande, R. A. (2020). Aspiration bronchopneumonia by Acinetobacter baumannii in a wildlife European hare (Lepus europaeus) in Brazil. Journal of Zoo and Wildlife Medicine, 51(1), 253–256. https://doi.org/10.1638/2019-0100

[11] López-Martín, M., Dubern, J. F., Alexander, M. R., & Williams, P. (2021). AbaM regulates quorum sensing, biofilm formation, and virulence in Acinetobacter baumannii. *Journal of Bacteriology, 203*(8), e00635-20. https://doi.org/10.1128/JB.00635-20

[12] Santhakumari, S., & Ravi, A. V. (2019). Targeting quorum sensing mechanism: An alternative anti-virulent strategy for the treatment of bacterial infections. *South African Journal of Botany, 120*, 81–86. https://doi.org/10.1016/j.sajb.2018.09.028

[13] Namasivayam, S. K. R., & Vivek, J. M. (2016). Screening of quorum sensing (QS) modulatory effect of medicinal plant extracts against quorum sensing mediated virulence factors of human pathogenic gram negative bacteria. *International Journal of Pharmacy and Pharmaceutical Research, 8*, 263. http://impactfactor.org/PDF/IJPPR/8/IJPPR,Vol8,Issue2,Article9.pdf

[14] Rivera, M. L. C., Hassimotto, N. M. A., Bueris, V., Sircili, M. P., de Almeida, F. A., & Pinto, U. M. (2019). Effect of Capsicum frutescens extract, capsaicin, and luteolin on quorum sensing regulated phenotypes. *Journal of Food Science, 84*(6), 1477–1486. https://doi.org/10.1111/1750-3841.14648

[15] Cappuccino, J., & Welsh, C. (2017). *Microbiology* (11th ed.). Pearson Education. https://elibrary.pearson.de/book/99.150005/9781292175799

[16] Ling, T. K., Liu, Z. K., & Cheng, A. F. (2003). Evaluation of the VITEK 2 system for rapid direct identification and susceptibility testing of gram-negative bacilli from positive blood cultures. *Journal of Clinical Microbiology, 41*(10), 4705–4707. https://doi.org/10.1128/JCM.41.10.4705-4707.2003

[17] Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M., Marchant, R., & Banat, I. M. (2016). Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotechnology Letters, 38*(6), 1015–1019. https://doi.org/10.1007/s10529-016-2079-2

[18] Martins, M., Viveiros, M., Couto, I., Costa, S. S., Pacheco, T., Fanning, S., Pagès, J. M., & Amaral, L. (2011). Identification of efflux pump-mediated multidrug-resistant bacteria by the ethidium bromide-agar cartwheel method. *In Vivo, 25*(2), 171–178. https://pubmed.ncbi.nlm.nih.gov/21471531/

[19] Mishra, B., Patel, B. B., & Tiwari, S. (2010). Colloidal nanocarriers: A review on formulation technology, types and applications toward targeted drug delivery. *Nanomedicine: Nanotechnology, Biology, and Medicine, 6*(1), 9–24. https://doi.org/10.1016/j.nano.2009.04.008

[20] Morovat, T., Bahram, F., Mohammad, E., Setareh, S., & Mohamad Mehdi, F. (2009). Distribution of different carbapenem-resistant clones of Acinetobacter baumannii in Tehran hospitals. *New Microbiologica, 32*(3), 265–271. https://pubmed.ncbi.nlm.nih.gov/19845108/

[21] Sun, X., Ni, Z., Tang, J., Ding, Y., Wang, X., & Li, F. (2021). The abaI/abaR quorum sensing system effects on pathogenicity in Acinetobacter baumannii. *Frontiers in Microbiology, 12*, 679241. https://doi.org/10.3389/fmicb.2021.679241

[22] Woodford, N., Ellington, M. J., Coelho, J. M., Turton, J. F., Ward, M. E., Brown, S., et al. (2006). Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. *International Journal of Antimicrobial Agents, 27*(4), 351–353. https://doi.org/10.1016/j.ijantimicag.2006.01.004

[23] Nadkarni, M. A., Martin, F. E., Jacques, N. A., & Hunter, N. (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology, 148*, 257–266. https://doi.org/10.1099/00221287-148-1-257

[24] Cobellis, G., Yu, Z., Forte, C., et al. (2016). Dietary supplementation of *Rosmarinus officinalis* L. leaves in sheep affects the abundance of rumen methanogens and other microbial populations. *Journal of Animal Science and Biotechnology, 7*, 27. https://doi.org/10.1186/s40104-016-0086-8

[25] Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocols, 3*(6), 1101–1108. https://doi.org/10.1038/nprot.2008.73

[26] George, D., & Mallery, P. (2019). *IBM SPSS Statistics 26 Step by Step: A Simple Guide and Reference* (16th ed.). Routledge. https://doi.org/10.4324/9780429056765

[27] A Abdul Kareem, M., & Mubarak, I. (2024). Activity of Ciprofloxacin and Resveratrol on Expression of AdeI J Genes in Clinical Acinetobacter baumannii Isolates. Academic Science Journal, 2(3), 139-160. https://dx.doi.org/10.24237/ASJ.02.03.793B

[28] Sharmin, N., Khoda, M. M. E., Uddin, M. N., Shamsuzzaman, S., & Jahan, H. (2024). Prevalence, antibiotic resistant pattern and genotypic detection of Acinetobacter baumannii isolated from different clinical specimens of patients admitted at a tertiary care hospital in Bangladesh. *Bangladesh Journal of Medical Microbiology, 18*(1), 37–43. https://doi.org/10.3329/bjmm.v18i1.77097

[29] Shaymaa Majeed AL–Dahlaki and Hadi R. Rasheed Al-Taai (2021) Gene expression of hcp gene in carbapenems resistance in Acinetobacter baumannii. Biochem. Cell. Arch. 21(1), 783-789. DocID: https://connectjournals.com/03896.2021.21.783

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

[30] AL-Maawi, I. T., & Al-Taai, H. R. (2026). Molecular Typing by Clonal Genetic Linkage among Carbapenem-Resistant Acinetobacter baumannii Isolated. Egyptian Journal of Medical Microbiology, 35(2),19-28. https://doi.org/10.21608/ejmm.2025.399233.1748

[31] Alwohaili, D. H. K., & Al-Awade, H. A. R. (2025). Isolation and identification of Acinetobacter baumannii from different clinical sources and determination of antibiotic resistance in Karbala city. *Karbala Journal of Pharmaceutical Sciences, 16*(26), 12–23. https://doi.org/10.62472/kjps.v16.i26.12-23

[32] Hadi, H., & Al Sammak, E. (2024). Efflux pumps adeA and adeB genes in clinical multidrug resistant (MDR) Acinetobacter baumannii strains in Mosul/Iraq. *Microbes and Infectious Diseases*. https://doi.org/10.21608/mid.2024.318528.2196

[33] Alwashaish, M. M., Aburowais, A. S., & Elmeheishi, F. M. (2025). Correlation between efflux pump activity, biofilm formation, and carbapenem resistance in clinical isolates of Acinetobacter baumannii from Libyan hospitals. *Journal of Microbiology and Infectious Diseases*, 15(4): 202-215. https://doi.org/10.5455/JMID.2025.v15.i4.8

[34] Tripathi, P., Banerjee, G., Gupta, M. K., Saxena, S., & Ramteke, P. W. (2013). Assessment of phylogenetic affiliation using 16S rRNA gene sequence analysis for Pseudomonas aeruginosa in patients with lower respiratory tract infection. The Indian Journal of Medical Research, 138(4), 557–561. https://pubmed.ncbi.nlm.nih.gov/24434264/

[35] Al-Khafaji, A. M. K., & Al-Fatlawy, H. N. K. (2025). Molecular genotyping of gyrA, blaOXA-51, and blaOXA-23 genes in multidrug-resistant Acinetobacter baumannii isolated from childhood asthma patients in Iraq. *Egyptian Journal of Medical Microbiology, 34*(4), 483–489. https://doi.org/10.21608/EJMM.2025.386369.1658

[36] Abdullah, R. M., & Mahdi, A. A. (2023). Studying the gene expressions of blaOXA-51-like and blaOXA-23-like in Acinetobacter baumannii. *Ibn Al-Haitham Journal for Pure and Applied Sciences, 36*(4), 32–39. https://doi.org/10.30526/36.4.3073

[37] Tang, J., Chen, Y., Wang, X., Ding, Y., Sun, X., & Ni, Z. (2020). Contribution of the AbaI/AbaR quorum sensing system to resistance and virulence of Acinetobacter baumannii clinical strains. *Infection and Drug Resistance, 13*, 4273–4281. https://doi.org/10.2147/IDR.S276970

[38] Ali, G., & Mohammad, G. (2024). Detection of abaI/abaR genes in Acinetobacter baumannii and phenotypic demonstration of N-acyl homoserine lactone. *Microbes and Infectious Diseases*. https://doi.org/10.21608/mid.2024.303775.2072

[39] Salih, S. M., & Al-Asady, Z. H. (2022). Molecular detection of abaI genes in Acinetobacter baumannii isolated from clinical specimens in some Iraqi hospitals. *Indian Journal of Forensic Medicine & Toxicology, 16*(2), 366–373. https://doi.org/10.37506/ijfmt.v16i2.17991

[40] Anbazhagan, D., Mansor, M., Yan, G. O., Md Yusof, M. Y., Hassan, H., & Sekaran, S. D. (2012). Detection of quorum sensing signal molecules and identification of an autoinducer synthase gene among biofilm forming clinical isolates of Acinetobacter spp. *PLoS One, 7*(7), e36696. https://doi.org/10.1371/journal.pone.0036696

[41] Kaur, A., Sharma, P., & Capalash, N. (2018). Curcumin alleviates persistence of Acinetobacter baumannii against colistin. *Scientific Reports, 8*, 11029. https://doi.org/10.1038/s41598-018-29291-z

[42] Landersdorfer, C. B., Ly, N. S., Xu, H., Tsuji, B. T., & Bulitta, J. B. (2013). Quantifying subpopulation synergy for antibiotic combinations via mechanism-based modeling and a sequential dosing design. *Antimicrobial Agents and Chemotherapy, 57*(5), 2343–2351. https://doi.org/10.1128/AAC.00092-13

[43] Kali, A., Bhuvaneshwar, D., Charles, P. M., & Seetha, K. S. (2016). Antibacterial synergy of curcumin with antibiotics against biofilm-producing clinical bacterial isolates. *Journal of Basic and Clinical Pharmacy, 7*(3), 93–96. https://doi.org/10.4103/0976-0105.183265

[44] Betts, J. W., Sharili, A. S., La Ragione, R. M., & Wareham, D. W. (2016). In vitro antibacterial activity of curcumin–polymyxin B combinations against multidrug-resistant bacteria associated with traumatic wound infections. *Journal of Natural Products, 79*(6), 1702–1706. https://doi.org/10.1021/acs.jnatprod.6b00286

[45] Shahverdi, A., Moghaddam, K., Iranshahi, M., & Yazdi, M. (2009). The combination effect of curcumin with different antibiotics against Staphylococcus aureus. *International Journal of Green Pharmacy, 3*, 141. https://doi.org/10.4103/0973-8258.54906

DOI: 10.69946/ojms/2026.04.01.07, pp(62-76)

[46] Dai, C., Lin, J., Li, H., Shen, Z., Wang, Y., Velkov, T., & Shen, J. (2022). The natural product curcumin as an antibacterial agent: Current achievements and problems. *Antioxidants, 11*(3), 459. https://doi.org/10.3390/antiox11030459

[47] Cordell, G. A., & Araujo, O. E. (1993). Capsaicin: Identification, nomenclature, and pharmacotherapy. *The Annals of Pharmacotherapy, 27*(3), 330–336. https://doi.org/10.1177/106002809302700316

[48] Wrynn, A. F. (2023). An overview of penicillin allergies for nurses. *Nursing, 53*(5), 27–31. https://doi.org/10.1097/01.NURSE.0000923664.66265.a4

[49] Andrade, R. J., & Tulkens, P. M. (2011). Hepatic safety of antibiotics used in primary care. *Journal of Antimicrobial Chemotherapy, 66*(7), 1431–1446. https://doi.org/10.1093/jac/dkr159

[50] Özçelik, B., Kartal, M., & Orhan, I. (2011). Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharmaceutical Biology, 49*(4), 396–402. https://doi.org/10.3109/13880209.2010.519390

[51] Santos, C. A., Lima, E. M. F., Franco, B. D. G. M., & Pinto, U. M. (2021). Exploring phenolic compounds as quorum sensing inhibitors in foodborne bacteria. *Frontiers in Microbiology, 12*, 735931. https://doi.org/10.3389/fmicb.2021.735931

[52] Akyuz, L., Kaya, M., Mujtaba, M., Ilk, S., Sargin, I., Salaberria, A. M., Labidi, J., Cakmak, Y. S., & Islek, C. (2018). Supplementing capsaicin with chitosan-based films enhanced anti-quorum sensing, antimicrobial, antioxidant, transparency, elasticity, and hydrophobicity. *International Journal of Biological Macromolecules, 115*, 438–446. https://doi.org/10.1016/j.ijbiomac.2018.04.040

[53] Patel, K., Panchal, R., Sakariya, B., Gevariya, M., Raiyani, R., Soni, R., & Goswami, D. (2025). Combatting antibiotic resistance by exploring the promise of quorum quenching in targeting bacterial virulence. *Microbe, 6*, 100224. https://doi.org/10.1016/j.microb.2024.100224

[54] Alum, E. U., Gulumbe, B. H., Izah, S. C., Uti, D. E., Aja, P. M., Igwenyi, I. O., & Offor, C. E. (2025). Natural product-based inhibitors of quorum sensing: A novel approach to combat antibiotic resistance. *Biochemistry and Biophysics Reports, 43*, 102111. https://doi.org/10.1016/j.bbrep.2025.102111

[55] Sethupathy, S., Prasath, K. G., Ananthi, S., Mahalingam, S., Balan, S. Y., & Pandian, S. K. (2016). Proteomic analysis reveals modulation of iron homeostasis and oxidative stress response in Pseudomonas aeruginosa PAO1 by curcumin inhibiting quorum sensing regulated virulence factors and biofilm production. *Journal of Proteomics, 145*, 112–126. https://doi.org/10.1016/j.jprot.2016.04.019

[56] Bahari, S., Zeighami, H., Mirshahabi, H., Roudashti, S., & Haghi, F. (2017). Inhibition of Pseudomonas aeruginosa quorum sensing by subinhibitory concentrations of curcumin with gentamicin and azithromycin. *Journal of Global Antimicrobial Resistance, 10*, 21–28. https://doi.org/10.1016/j.jgar.2017.03.006

[57] Jeong, G. J., Khan, F., Khan, S., Tabassum, N., Mehta, S., & Kim, Y. M. (2023). Pseudomonas aeruginosa virulence attenuation by inhibiting siderophore functions. *Applied Microbiology and Biotechnology, 107*(4), 1019–1038. https://doi.org/10.1007/s00253-022-12347-6

[58] Diaz-Guerrero, M., López-Jácome, L. E., Franco-Cendejas, R., Coria-Jiménez, R., Martínez-Zavaleta, M. G., González-Pedrajo, B., Huelgas-Méndez, D., & García-Contreras, R. (2025). Curcumin inhibits type III secretion of Pseudomonas aeruginosa. *PeerJ*. https://doi.org/10.7717/peerj.19725.