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Activity of Ciprofloxacin and Resveratrol on Expression of *AdeI J* Genes in Clinical *Acinetobacter baumannii Isolates*

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Abstract

Acinetobacter baumannii considered one important pathogenic bacteria and responsible for several nosocomial and community infections for human and , possess the ability to form biofilms , and continued development of antibiotic resistance to drugs used to treat bacterial infections .The current study was performed ,during the period 1/9 /2022 to the end of December 30 /12 /2022 , a total of 210 blood specimens were collected in Baqubah Teaching Hospital /Iraq to investigate the prevalence of bacterial isolates among patients in Intensive Care Unit (ICU) , detect antibiotic resistance , biofilms formation , molecular detection of efflux pumps *adeI J* genes , and activity of ciprofloxacin and resveratrol on expression of these two genes .The results revealed that the percentage of positive growth of *A .baumannii*, which isolates from blood samples with among ICU patients were 12 %, presence of the highest resistance of 25 isolates against fourteen types of antibiotics, our finding showed significant differences before and after treatment of resistance isolates with Resveratrol with a higher reduction expression of *AdeIJ* genes in all isolates under study reaching 0.59 and 0.28, respectively.

Keywords: *A .baumannii*, *AdeI J genes* , Antibiotic , Efflux pump inhibitors .

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فعالية السبروفلوكساسين والريسفيراترول على التعبير الجيني للجينين *adeI,J* في العزلات السريرية للراكدة البومانية

منتهى احمد عبد الكريم و كريم ابراهيم مبارك

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الخلاصة

تعتبر *Acinetobacter baumannii* من البكتيريا المهمة المسببة للأمراض والمسؤولة عن العديد من الإصابات للإنسان في المجتمع والمستشفيات اذ تمتلك العديد من عوامل الفوعة منها : القدرة على تكوين الأغشية الحيوية ، والتطور المستمر لمقاومة العزلات لمضادات الحياة التي تستخدم في علاج الاصابات التي تسببها البكتريا .أجريت الدراسة الحالية خلال الفترة من 1 / 9 /2022 الى 30 /12 /2022 , تم جمع مائتين وعشرة عينات دم من المرضى الراقدين في وحدة العناية المركزة في مستشفى بعقوبة التعليمي / العراق لبحث انتشار العزلات البكتيرية و مقاومة تلك العزلات لمضادات الحياة , تكوين الأغشية الحيوية , والكشف الجزيئي لجينات مضخات الدفق *AdeIJ* , وفعالية السبروفلوكساسين والريسفيراترول على التعبير الجيني .أظهرت النتائج أن نسبة النمو الموجب لعزلات ال *A .baumannii* من عينات الدم للمرضى كانت (12٪) , وابد ت العزلات نسب مقاومة عالية ضد أربعة عشر نوعًا من المضادات المستخدمة , كما أظهرت النتائج فرق معنوي في التعبير الجيني قبل وبعد المعاملة للعزلات بال Resveratrol مع انخفاض عال في التعبير للجينين *AdeIJ* في العزلات قيد الدراسة حيث بلغ 0.59 و 0.28 على التوالي .

الكلمات المفتاحية *A .baumannii* : , جينات , *AdeI J* مضادات الحياة , مثبطات مضخات الدفق .

Introduction

Acinetobacter baumannii (*A .baumannii*) is a gram negative cocci bacillus, late to lactose fermenting, oxidase negative, aerobic and human opportunistic extracellular pathogen originating from hospital acquired infections to be also known as a nosocomial infection .*A .baumannii*, also known as “Iraqibacter ”because it first appeared in US military treatment facilities center in Iraq, has rapidly increased and become one of the most troublesome pathogens for healthcare institutions globally and currently tops [1,2] .Several epidemiological studies have reported the occurrence of Multi Drug Resistance (MDR) .*A .baumannii* infections in variety regions of the world including Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan and Korea [3] .

Bacteria are primarily considered a healthcare-associated pathogen and many reports indicated that bacteria are the primary cause of nosocomial infections including septicemia, bacteremia, ventilator-associated pneumonia, wound sepsis, endocarditis, meningitis, and urinary tract infections [4] *A .baumannii* causes bloodstream infections in Intensive Care Unit (ICU) among patients and frequently linked with central venous catheters, mechanical ventilation, pneumonia, drain use, and respiratory and cardiovascular failure [5 ,6] organism are opportunistic have been account for up to 2% of all bloodstream infections, and are commonly associated with intravascular devices [7].

Resistance of bacteria to antibiotics which has increased .Efflux pumps are one important mechanisms of bacterial resistance to most antimicrobial that used for treatment of different bacterial infection [8] .Recent studies with the relation of antibiotic resistance concentrated to investigating the effect of different agents to obtained useful efflux pumps inhibitors, according to the results and conclusions of this research showed that some molecules (natural or chemical) which have the capacity to act specifically on the efflux system to restore the action of antibiotics and commonly called as efflux pumps inhibitors (EPIs) [9] .

Resveratrol is a natural polyphenolic substance from grape skin and seeds [10] .It is a phytoalexin belonging to the polyphenol still bends group with considerable strong antimicrobial activity against various human diseases [11] .Furthermore, resveratrol is safe for humans and has been used as a food preservative due to its antimicrobial and anti-biofilm characteristics against food-borne poison [12,13] .The study aimed to isolation *A .baumannii* from blood specimens collected from (ICU) patients, detect the ability of isolates to biofilms formation, conduct molecular analysis of (*Ade I J*) efflux pumps genes, and investigate the effect of ciprofloxacin, resveratrol and their combination on the expression of two efflux pumps genes .

Materials and Methods

Identification of *A .baumannii*

Two hundred ten blood specimens were collected from patients admitted to the Intensive Care Unit (ICU) in Baqubah Teaching Hospital during the period between 1/ 9 /2022 to 30/ 12 / 2022 to investigate the prevalence of *A .baumannii*, some information related to patients were recorded included: name, age, gender, and type of infection .The specimen was cultured using BACT\Alert 3D system [14] .After the inoculation of the specimen by streaking onto MacConkey agar, the plates were incubated for 24 hrs at 37ºC .Expected colonies of *A .baumannii* were sub cultured on HiChromeTM Acinetobacter agar and incubated for 24 hrs at 37ºC .Morphological Identifications of growth isolates by Gram staining and Colony features [15] .Many biochemical tests include IMPIC, urease, oxidase test, hemolysin production, citrate utilization, ability to grow at 44 C were performed according to [16] .Confirmation of isolate identification carried by the viteik-2 compact system [17] and molecular detection of the blaOXA-51 gene [18].

Antibacterial Susceptibility Test

All *A .baumannii* isolates were tested according to the Kirby-Bauer disc diffusion method [19] to detect the antibiotic sensitivity of these isolates to fourteen antibiotics .The bacterial suspension was prepared from 24 hour age colonies at 1.5 x108 cell / ml for each isolate by comparing McFarland standards solution (0.5) .Then by using sterilized cotton swabs bacteria were spread on the surface of the Muller Hinton Agar plate .The plate was left to dry at room temperature for 15 minutes .Seven antibiotic discs were distributed on the surface of each agar plate by using sterilized forceps .Finally the plates were incubated period for 24 hours at 37°C .The inhibition zone measurement was documented in millimeters surrounding the disc, and the evaluation of the resistant and sensitive isolate was conducted following the guidelines set forth by the Clinical and Laboratory Standards Institute (CLSI 2022) [20].

The serial dilution method on Muller-Hinton Broth (MHB) with concentration serial dilution between 2–1024 ug/ml for each agent was used to determine the minimum inhibitory

concentration (MIC) of ciprofloxacin and resveratrol for three MDR isolates to examine their impact on gene expression .Bacterial suspension 1.5 x108 cell / ml was prepared as in the antibiotics susceptibility test above .The lowest antibiotic concentration that prevents bacterial growth was identified as the MIC following a 24-hour incubation period at 37°C [21].

Biofilm Detection of *A .baumannii*

Biofilm formation by 25 isolates was carried out in 96-well flat-bottom polystyrene micro plates using 0.1 % crystal violet [22] .A total of 20 ml of overnight bacterial culture was used to inoculate flat-bottom micro titer wells containing 180 ml of sterile brain heart infusion broth with 2% sucrose (0.5 gm sucrose/25 ml of the medium) .Control wells contained only 200 µl of brain heart infusion broth with 2% sucrose; the plate had been covered with a lid, sealed with a stripe of Para film, and incubated for 24 hours at 37°C .The wells were washed three times with normal saline (pH 7.2) to remove detached bacterial cells .The plate was dried at room temperature for 15 minutes, and 200 µl of crystal violet solution (0.1%) was added and left for 15 minutes .Crystal violet solution was removed carefully, and the wells were washed three times with distilled water to eliminate the unbounded dye and allowed to dry at room temperature .Two hundred microliters of 95% ethanol were poured into each well to extract the bound dye .The absorbance was determined at 630 nm using an ELISA reader .The optical density (OD) value for the control well was deducted from the sample values .When the absorption of the cultivated pit was compared with the control pits, the result follows as follows: If OD≤ ODc (Considered non-biofilm producer); if ODc ≤ OD ≤2*ODc (Considered produce biofilm at moderate level); if 2*ODc ≤ OD(Considered produce biofilm at strong level) .Where OD (Represented the tested isolates) and ODc (Represented control pits).

Molecular Study

The molecular study includes the detection of the *A .baumannii* identification gene blaOXA-51 and *AdeIJ* efflux pumps genes in eighteen isolates**.**

DNA extraction: Genomic DNA had been extracted from the bacteria isolates using a genomic DNA purification Kit supplemented by a manufactured commercial company (Promega, USA)

.DNA was purified and its purity was measured by using nano-drop spectrophotometer .Then genomic DNA was quantified and kept at -20°C .The PCR program included a denaturation step for 5min at(95) °C, followed by (1) cycles of annealing at (54 to 58°C) for 60 seconds according to primer design table (1), and a final extension at 72 °C for 5 minutes at (1) cycle .On gel electrophoresed the PCR products were detected**.**

Table 1: Primers with their Annealing Temperature and size

Primer Name	Sequence 5`-3`	Annealing Temp .(°C)	Product size (bp)	Referance
adeI-F	ATCGCGCTTGTTGGTTGTAG	54	541	[23]
adeI-R	AAGCACCAGCCGTTACTGAA			
adeJ-F	ATTGCACCACCAACCGTAAC	54	453	
adeJ-R	TAGCTGGATCAAGCCAGATA			
Oxa-51-like-F	TAATGCTTTGATCGGCCTTG	58	342	
Oxa-51-like-R	TGGATTGCACTTCATCTTGG			

Expression of efflux pumps genes *(AdeJ,I)*

The TRIzolTM reagent method was used to extract and purify the RNA from the sample .Following with the supplier's manual instructions, the concentration of the extracted RNA was determined using a QuantusTM Fluorometer (Promega, USA) .With the Gene 9600 Quantitative Instrument real-time PCR was run. .The primers were preparated for *adeI* and *adeJ* [23] .The produced solution was placed in Real time PCR Cycler to measure the Cycle Threshold [CT] value by heat reaction .RT-PCR is used for quantification of the levels of gene expression .The measured CT values during heat reaction are recorded on computer .The differences in gene expression levels were calculated by the ΔΔCT method according to [24]

ΔCT (test) = CT gene of interest (target, test) – CT internal control

ΔΔ CT=ΔCT (test)- ΔCT (calibrator), 2 -ΔΔCt = Normalized expression ratio

Statistical Analysis

Data were analyzed using SPSS version 23 (Statistical Package for Social Science, Chicago, IL, USA), a statistical analysis program .For the comparison of categorical data, Chi-square

was used in the data analysis .Data were presented as means and standard deviation; differences were judged significant when the p-value was less than 0.05.

Results and Discussion

Distribution of *A .baumannii* isolates

The result of growth showed that twenty five (11.90 %) isolates of *A.baumannii* were detected from total of 210 blood specimens were collected from ICU patients .The percentages of *A .baumannii* based on disease type equal to 4 (16 %) for burn infections , 5 (20%) for surgical wounds , 6 (24%) for diabetes foot (gas gangrene), 3(12%) for brain bleeding , 4 (16 %) for respiratory tracts , 3(12%) and for renal failure .Figure (1) .This result of the current study was nearly to result by [25] with a ratio (10.14%).

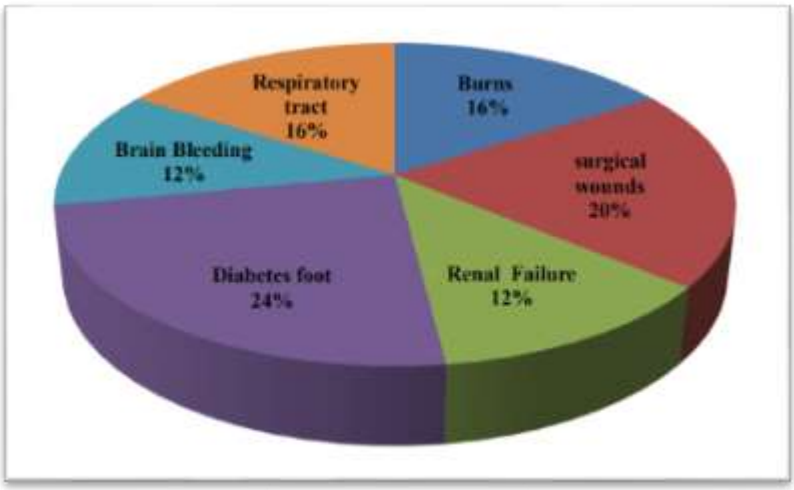

Figure 1: *A.baumannii* isolates were distributed among the different infection categories in the intensive care unit

Identification of *A.baumannii*

All blood specimens (210) were cultured using the BACT\Alert 3D system [14], and then the specimens inoculated by streaking on MacConkey agar were incubated for 24 hrs at 37ºC .The growth colonies of *A.baumannii* appeared pale, small, pink, and non-lactose fermented on MacConkey agar .[26] Expected bacteria colonies were sub-cultured on HiChromeTM

Acinetobacter agar colonies appear on the last medium light purple with a halo around the colonies [27] .While on Blood, agar colonies showed non-pigmented creamy, convex mucoid with a smooth surface in diameter 0.5_2 mm, and the colonies had productive hemolysin .Gram's stain showed that of *A .baumannii* Gram-negative with various shapes according to the stage of growth phase from bacilli to cocco-bacilli .Diplococcus and some had short chains .*A.baumannii* can grow on 44 ºC [28].

The results of biochemical tests reveal that all the isolates of *A.baumannii* showed positive results for the catalase test because the bacteria can create the catalase enzyme, However, all isolates give negative reaction for Oxidase test Lactose fermenter , producinge Indol , Methyl red and Voges- Proskauer, .The positive results were showed in Simmons citrate, while the urease test gave variable results, all isolates were be identified by using the viteik-2 compact system .

Molecular identification of eighteen selected isolates by Polymerase chain reaction showed that all isolates gave positive results for blaOXA-51 gene indicating that the isolates (100%) diagnosed as A .baumannii , figure (2) , the blaOXA-51 gene were considered a specific gene to the species and intrinsic chromosomally located gene that was diagnostic of A .*baumannii*, and also consider a better molecular marker than the 16s rRNA gene for the study of phylogenetic and taxonomic relationships at the species level precisely for this bacterium [18] .The detection by *blaOXA-51* gene provide an accurate and practical method to identifying *A .baumannii*, and would be more reliable than biochemical identification, which is most extremely useful .Since *A .baumannii* is clinically the most significant of the Acinetobacter species, the ability to distinguish it rapidly from other members of the genus would be highly valuable [29] .The finding of the present study is consistent with the results of those studies by [30].

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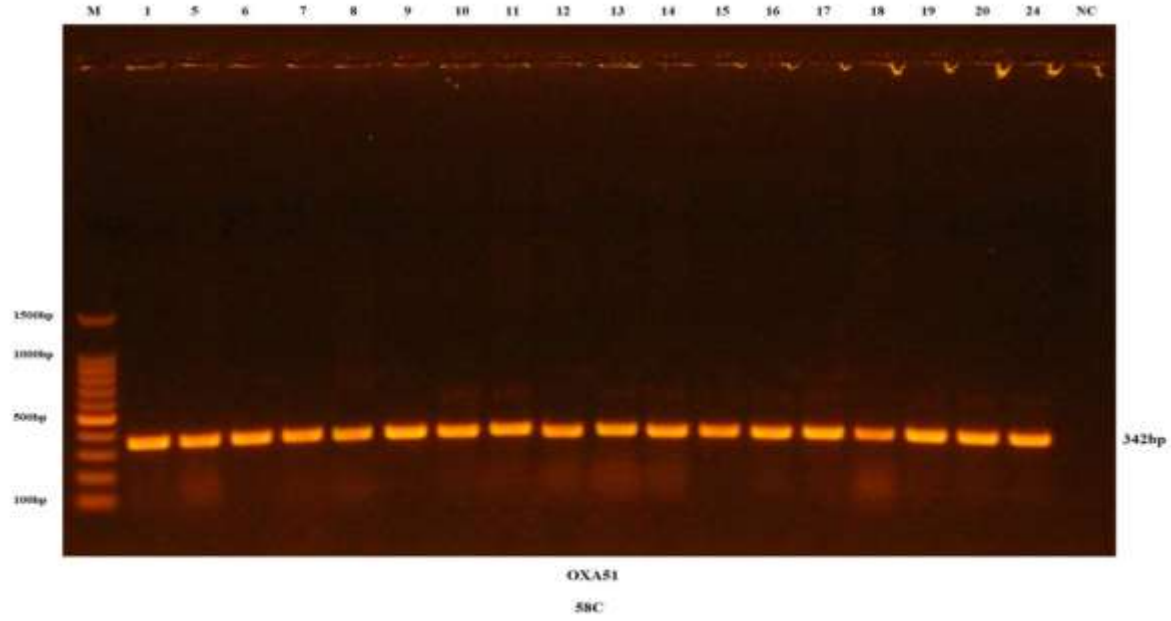

Figure 2: *A .baumannii* samples were fractionated on a 1.5% agarose gel electrophoresis and stained with Eth .Br to represent the outcomes of *bla- OXA51* gene amplification.100 bp ladder marker for .lanes 1–24 .The 342bp PCR product

Antibiotics Resistance of *A .baumannii*

The finding of the present study in table (2) revealed that a high percentage of A .baumannii isolates were resistant to Cefotaxime with a ratio (of 100%), Ticarcillin- clavulante and Ceftriaxone with a ratio of 96% all these results were similar to those study by [31] .The resistance ratio to piperacillin- tazobactam, cefepime, and tobramycin was (92%) .This is consistent with the study of [31] *.A.baumannii* shows resistance to fluoroquinolones class (ciprofloxacin, levofloxacin) with a percentage of 72% .The findings of [32] were similar to the results of our study .Choice drugs of treatment, imipenem and meropenem, showed resistance with 72% and 76%, respectively .The agreement with [33] obtained the same results .But, the results disagree with [31] .doxycycline showed low resistance with percentage (64 %) These results were near the study of [34] .Amikacine, tetracycline, and trimethoprim sulfomethazol showed resistance with ratios (of 84% and 80%) respectively, this finding reported with [35] .

Table 2: Proportion of *A .baumannii* isolates and antibiotic resistance

ANTIBIOTICS TYPE	RESISTANT NO & (%)	ANTIBIOTICS TYPE	RESISTANT NO& (%)
Piperacillin-tazobactam	23 (92)	**Tobramycin**	23 (92)
Ticarcillin-clavulante	24 (96)	**Amikacine**	22 (88)
Cefepime	23 (92)	**Tetracycline**	21 (84)
Cefotaxime	25 (100)	**Doxycycline**	16 (64)
Ceftriaxone	24 (96)	**Ciprofloxacin**	18 (72)
Imipenem	18 (72)	**Levofloxacin**	18 (72)
Meropenem	19 (76)	**Trimethoprim – sulfamethoxazol**	20 (80)

Clinical isolates of *A .baumannii* can classified depending on their resistant patterns [36] into three major groups where their percentage of antibiotics resistance represent (32 %) Multi Drug Resistant (MDR), (16 %,) Extensive Drug Resistant, (XDR) and (52 %) for Pan Drug Resistant (PDR) , this result agreement with [37] .While different from the result of [31] ,figure (3).

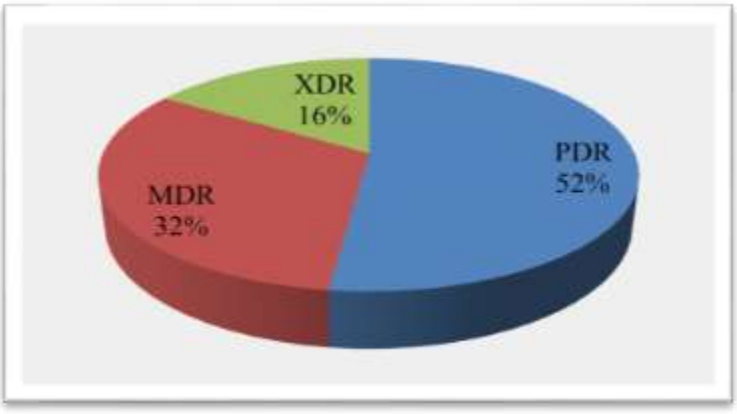

Figure 3: The percentage of MDR, XDR and PDR of *A .baumannii* Isolates

Minimum Inhibitory Concentration (MIC)

Table (3) shows the value of Minimum Inhibitory Concentration for ciprofloxacin for all three MDR *A .baumannii* isolates (256 ug / ml) .This was acceptable with [27] being considered as highly resistant .While mic values of resveratrol ranged from (32- 256 ug /ml) the results of the present study agreement with result by ([38] who wrote that mic of resveratrol ranging

between (32-128 ug /ml) and result of current study was nearly to [39] who reported mic of resveratrol ranging from (32-512).

Table 3: MIC and sub MIC values of Ciprofloxacin and Resveratrol for three MDR isolates of *A.baumannii*

SERIES .OF ISOLATES	SUB MIC OF CIPROFLOXACIN UG/ML	MIC OF CIPROFLOXACIN UG/ML	SUB MIC OF RESVERATROL UG/ML	MIC OF RESVERATROL UG/ML
A.b 14	128	256	32	64
A.b15	128	256	16	32
A.b24	128	256	128	256

Biofilm formation of *A .baumannii*

The majority of isolates of *A .baumannii* develop biofilm formation .*A .baumannii* isolates appeared strongly biofilm, with 15 (60%) strongly biofilm , while 10 (40%) isolates were moderately biofilm producers .Table (4) .These results are in agreement with studies by [40], which was (63 %) for strongly adherent biofilm forming .The current result is not acceptable with another study by [41], who reported that *A .baumannii* could produce a strong biofilm of isolates with a ratio (of 80%) , the differences between the findings of the present research and the previous research may be due to the different components utilized during the process, such as the using TSA or N.B media, microbial concentration or incubation period (24 hours), as the density of the cells in the biofilm increases as the incubation period increases, In addition, the dye concentration effects on the results as the concentration of 0.5 percent gives better results when compared to 1% [42].

Table 4: Percentage of biofilm formation of (25) *A .baumannii* isolates

BIOFILM DEGREE	NUMBER	PERCENTAGE	RANGE OF ABSORBENCY AT 630 NM
Strong	15	60	0.287 - 0.512
Moderate	10	40	0.144 - 0.261
Non formation	zero	Zero	----------
Control	----------	----------	0.134

A micro titer plate is An essential tool for studying the early stages of biofilm development [43] .This approach has the advantage of an economical quantitative technique for identifying serious factors and standard conditions of culture for in vitro forming biofilms .It has a 100 percent higher sensitivity, and it has been utilized as a standard technique for quick access to cell attachment and biofilm formation in a variety of gram-positive and negative bacteria [44], differences in biofilm formation among clinical isolates, in association with the epidemic spread of strains and the severity of infections [45, 40].

Molecular Study Of Efflux Pump (*Ade IJ) genes*

The results of detection on RND-efflux pump (*Ade I J) genes* in *A .baumannii* as shown in figure (4 ,5) which appeared the genes of RND superfamily efflux pump in *A .baumannii* were distributed in a worldwide and were found in approximately 100% of clinical strains .These findings are acceptable with [46, 47],who both found in their studies that super family RND *(AdeI J)* genes were most frequent and found in almost all isolates with a percentage (100%) .This result is similar to the finding of [48,49], who indicates the presence of *AdeIJ* genes in each strain of A .baumannii .by (90-100%).

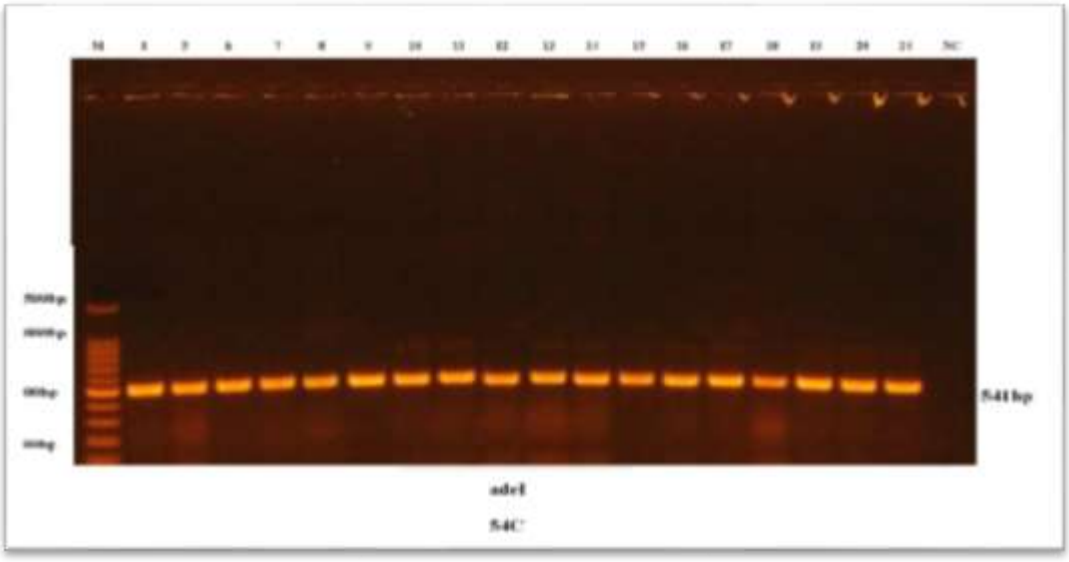

Figure 4: Showed *A .baumannii* samples were fractionated on a 1.5% agarose gel electrophoresis stained with Eth.Br .M: 100bp , Lanes 1-24 resemble 541bp PCR products

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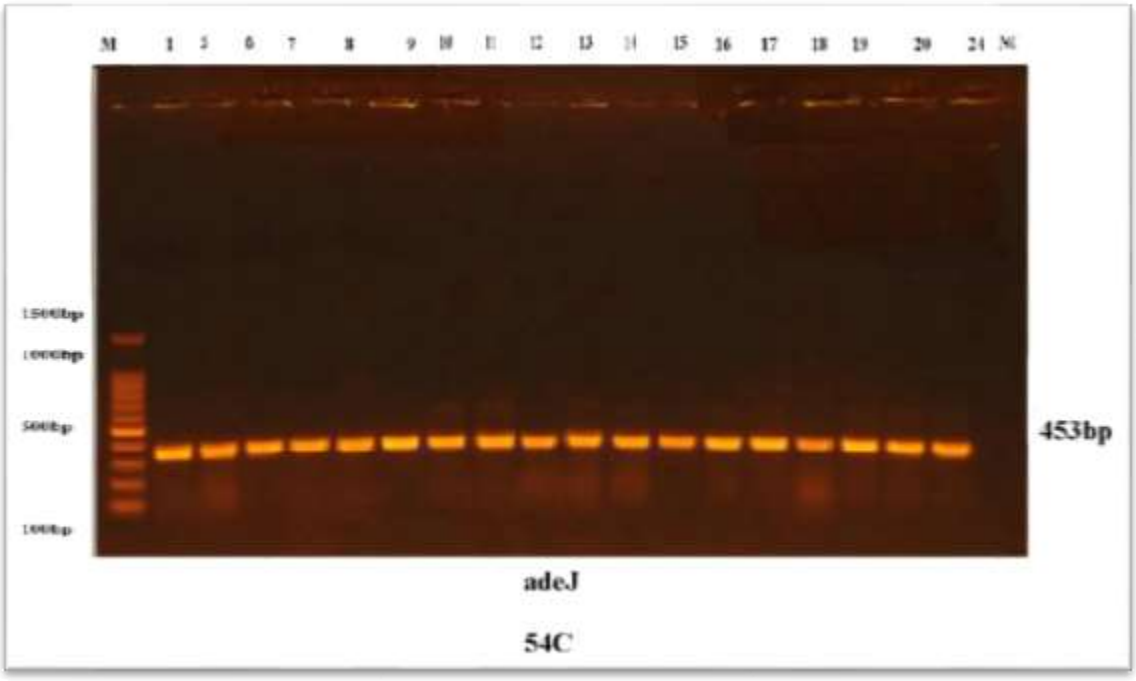

Figure 5: Appeared amplification *of AdeJ gene of A .baumannii* isolates were separated on 1.5% agarose gel electrophoresis stained with Eth.Br .M: 100bp ladder marker .Lanes 1-24 resemble 453bp PCR products.

Activity of Ciprofloxacin &Resveratrol on Expression *of adeI* genes:

The minimum inhibitory concentration of three MDR resistance *A .baumannii* isolates that were used in the gene expression study were ciprofloxacin (256 ug/ ml) and resveratrol (64, 32, 256 ug/ ml) .The results were presented in Table (5) showed an increase in the expression of *adeI* where the folding mean (1.35) after treated with ciprofloxacin resulted in the noticeable effort of bacteria for survival and growth .The decreased expression of *adeI* showed after treatment with resveratrol (0.59) and a combination of ciprofloxacin & resveratrol (0.79) .This result is beneficial in changing antibiotic resistance because resveratrol increased ciprofloxacin susceptibility with increased intracellular accumulation of ethidium bromide in *A .baumannii,* indicating that resveratrol acts as an efflux pump inhibitor and has synergistic bactericidal effects when combined with ciprofloxacin against resistant clinical isolates of *A .baumannii* [50] .The statistical analysis also revealed significant differences (P-values 0.033) when

comparing treated and untreated isolates .The result of this study reveals that a combination of ciprofloxacin with resveratrol was much more effective than ciprofloxacin for reducing the expression of the added gene and, as a result, increased the ability of the drugs to kill the bacteria or achieving a synergistic effect best more than the antibiotics when utilized alone [51] .This result is acceptable to the work of [52], who indicates overexpression of the *adeI* gene, which plays a significant role in resistance to antibiotics and *adeABC* genes of the RND-efflux pump.

Table 5 : Expression of *adeI* gene before and after treatment with CIP &R

ISOLATE	*16SRRNA*	*ADEI*	ΔCT	ΔΔCT	FOLDING	MEAN	*P –VALUE*
A .b 14	13.95	12.89	-1.07	0.00	1.00	1	0.033
A .b 15	18.57	17.79	0.78	0.00	1.00		
A .b 24	13.59	12.45	-1.14	0.00	1.00		
A .b 14 (CIP)	15.73	15.91	0.18	1.24	0.42	1.35	
A .b 15 (CIP)	18.22	15.59	-2.63	-1.85	3.01		
A .b 24 (CIP)	16.62	16.12	-0.50	0.64	0.64		
A .b 14 (R)	15.60	15.29	0.31	0.75	0.59	0.59	
A .b 15 (R)	16.02	16.11	0.09	0.87	0.55		
A .b 24 (R)	18.72	18.17	0.42	0.81	0.65		
A .b 14 (CIP +R)	15.73	14.53	1.20	0.14	0.91	0.79	
A .b 15 (CIP+ R)	18.22	16.85	1.38	0.60	0.66		
A .b 24 (CIP+ R)	16.62	15.17	1.46	0.31	0.80		

A .b = *A .baumannii* , CIP = Ciprofloxacin , R = Resveratrol

Activity of Ciprofloxacin & Resveratrol on expression of *adeJ* gene

According to the results of the current study in Table (6) .The folding mean values after treatment with Sub MIC of ciprofloxacin, Sub MIC of resveratrol, and a combination of(0.25 MIC) of ciprofloxacin and (0.25 MIC) resveratrol were 0.28, 0.43, 1.57, respectively .The statistical analysis also revealed significant differences (*P*-values 0.43) when comparing treated and untreated isolates .The high level of expression of added in *A.baumannii* isolates at level 1.57 may be induced by the ingestion of antibiotics, leading to a significant increase in expression levels that enhanced their ability of these isolates to release a large number of antibiotics, thereby allowing them to confer more resistance to ciprofloxacin [49] .The bacteria develop different mechanisms of resistance to block the antibacterial drug inhibitory or bactericidal effects targeted by one or more of these capacities .The primary mechanism is the

over-expression of efflux pump systems, which consists of extruding antibacterial molecules out of the bacterial cell, thereby decreasing their concentrations to an insufficient level for a demonstrated impact .It is the main mechanism giving rise to MDR .Efflux pumps are membrane-spanning proteins found in both prokaryotic and eukaryotic cells in cytoplasmic membranes .They are active transporters, meaning they need a source of chemical energy [53] .This result in agreement with the results of [47, 49], both indicate that *AdeJ* plays a significant role in supporting antibiotic resistance . High-level RND efflux pump expression is commonly observed in Gram negative MDR clinical isolates [52], the reduction in expression of (*ade I,J)* .The genes after bacterial isolates treatment with resveratrol indicates that natural agents (from plant extracts) may be useful for inhibiting efflux pumps and making bacteria more susceptible to antibiotics .Resveratrol can change bacteria pathogenicity , reduce membrane integrity, and prevent biofilm formation by interfering with quorum sensing [54] .Recent media emphasis has focused on the antioxidant, anti-aging, and anti-cancer properties of resveratrol .In addition to their function resveratrol has been linked to antibacterial activity against food pathogens [55] .The efflux pump performance in *A .baumannii* may be inhibited in a variety of ways, according to the processes implicated in these systems' inhibition: reduced expression of the genes that code for the efflux pump alteration of drug structure, blocked of efflux-related inner or outer membrane proteins, blockage of efflux pumps employing inhibitors, disruption of pump assembly, disruption of proton motive force [56] .

Table 6: Expression of *adeJ* gene before and after treatment with CIP & RV

ISOLATE	16SRRNA	ADEJ	ΔCT	ΔΔCT	FOLDING	MEAN	P –VALUE
A .b 14	13.95	15.08	1.13	0.00	1.00	1	0.043
A .b 15	18.57	18.63	0.06	0.00	1.00		
A .b 24	13.59	14.57	0.98	0.00	1.00		
A .b 14 (CIP)	15.73	18.36	2.63	1.50	0.35	0.43	
A .b 15 (CIP)	18.22	19.18	0.95	0.89	0.54		
A .b 24 (CIP)	16.62	18.93	2.31	1.33	0.40		
A .b 14 (R)	15.60	18.30	2.70	1.57	0.34	0.28	
A .b 15 (R)	16.02	18.89	2.87	2.81	0.14		
A .b 24 (R)	18.72	18.23	2.15	1.71	0.36		
A .b 14 (CIP +R)	15.73	17.06	-1.33	-0.20	1.15	1.57	
A .b 15 (CIP+ R)	18.22	19.57	-1.35	-1.29	2.44		
A .b 24 (CIP+ R)	16.62	18.84	-1.60	-0.62	1.35		

A .b = *A .baumannii* , CIP = Ciprofloxacin , R = Resveratrol

Conclusions

Prevalence of *Acinetobacter baumannii* among ICU patients at Baqubah Teaching Hospital were 12% with a high degree of antibiotic resistance .Molecular detection shows that all isolates of *A.baumannii* contain *adeI J* genes .Resveratrol alone causes a higher effect in the reduction of the expression of *adeIJ* genes than ciprofloxacin, and a combination of both ciprofloxacin and resveratrol indicates this substance is useful to act as inhibitors of efflux pump to minimize the antibiotic resistance in resistance of *A .baumannii* isolates.

References

1. I. Chapartegui-Gonzalez, M. Lazaro-Diez, Z. Bravo, J. Navas, J. M. Icardo, J. Ramos-Vivas, *Acinetobacter baumannii* maintains its virulence after long-time starvation, Public Library of Science One, 13(8), e0201961(2018)
2. M. Nguyen, S .G. Joshi, Carbapenem Resistance in *Acinetobacter baumannii*, and their Importance in Hospital-acquired Infections: A Scientific Review, Journal of Applied Microbiology, 131(6), 2715–2738(2021)
3. A. Z. Kanafani, S. S. Kanj, Ministry of Health, Kingdome of Saudi Arabia, (2014)
4. S. B. Al-masaudi, *Acinetobacter spp*. as nosocomial pathogens: Epidemiology and resistance features, Saudi journal of biological sciences, 25(3),586-596(2018)
5. N. Guo, W. Xue, D. Tang, J. Ding, B. Zhao, Risk factors and outcomes of hospitalized patients with blood infections caused by multi drug resistant *Acinetobacter baumannii* complex in a hospital of Northern China, Am J Infect Control, 44(4),e37-9(2016)
6. M. N. L. Kurihara, R. O. de Sales, K. E. da Silva, W. G. Maciel, S. Simionatto , Multidrug-resistant *Acinetobacter baumannii* outbreaks: a global problem in healthcare settings, Journal of the Brazilian Society of Tropical Medicine, 53,(e20200248), (2020)
7. J. Melnick, Adelbergs, Medical Microbiology, 28th .ed., (The McGraw-Hill Companies , Inc., New York, 2019), 258 -259

8. R. Kumar, S. Pooja Patial, A Review on Efflux Pump Inhibitors of Gram-Positive and Gram-Negative Bacteria from Plant Sources, Current Microbiology and Applied Sciences, 5, 837-855(2016)
9. M. J. Cheesman, A. Ilanko, B. Blonk, I. E. Cock, Developing New Antimicrobial Therapies: Are Synergistic Combinations of Plant Extracts/Compounds with Conventional Antibiotics the Solution, Pharmacognosy Reviews , 11, 57(2017)
10. L. Kuršvietienė, I. Stanevičienė, A. Mongirdienė, J. Bernatonienė, Multiplicity of effects and health benefits of resveratrol, Medicina, 52(3), 148-155(2016)
11. A. Duarte, A .C. Alves, S. Ferreira, F. Silva, F .C. Domingues, Resveratrol inclusion complexes: antibacterial and anti-biofilm activity against Campylobacter spp. and Arcobacter butzleri, Food Research International, 77, 244-250(2015)
12. S. Ferreira, F. Domingues, The antimicrobial action of resveratrol against Listeria monocytogenes in food-based models and its antibiofilm properties, Journal of the Science of Food and Agriculture, 96(13), 4531-4535(2016)
13. M .V. Selma, M. Larrosa, D. Beltrán, R. Lucas, J .C. Morales, F. Tomás-Barberán, J. C. Espín, Resveratrol and some glucosyl, glucosylacyl, and glucuronide derivatives reduce *Escherichia coli* O157: H7, Salmonella Typhimurium, and Listeria monocytogenes Scott A adhesion to colonic epithelial cell lines, Journal of agricultural and food chemistry, 60(30), 7367-7374(2012)
14. S. Rovelli, The role of polysaccharides in plasmodium falciparum malaria, (2010)
15. S. Constantiniu, A. Romaniuc, L .S. Iancu, R. Filimon, I. Taraşi, Cultural and biochemical characteristics of Acinetobacter spp. strains isolated from hospital units, The journal of preventive medicine, 12(3-4), 35-42(2004)
16. A. O. Darweesh, A. A. Shareef, F. A. A. Alriyahee, Isolation and Identification of Cellulytic Ruminant Pseudomonas Aeruginosa by Classical and Molecular Methods in Basrah Province, Annals of the Romanian Society for Cell Biology, 4672-80(2021)
17. T. Addis, Y. Mekonnen, Z. Ayenew, S. Fentaw, H. Biazin, Bacterial Uropathogens And Burden Of Antimicrobial Resistance Pattern In Urine Specimens Referred To Ethiopian Public Health Institute, Plos One, 16, E0259602(2021)

18. B. Trupti, G. S. Bhatambare, M. Varma, M. Pandey, Species-level identification of Acinetobacter by 16s rRNA sequencing: Necessity today, essentiality tomorrow, New Nigerian Journal of Clinical Research, 5, 55, (2016)
19. J .G. Cappuccino, C .T. Welsh, Microbiology: A Laboratory Manual, 11th ed (Pearson Education, England, 2018)
20. CLSI, Performance Standards for Antimicrobial Susceptibility Testing, 27th ed (Wayne, PA: Clinical and Laboratory Standards Institute, 2022)
21. A. Louie, D. L. Brown, W. Liu, R.W. Kulawy, M. R. Deziel, G. L. Drusano, In vitro infection model characterizing the effect of efflux pump inhibition on prevention of resistance to levofloxacin and ciprofloxacin in *Streptococcus pneumoniae*, Antimicrobial agents and chemotherapy, 51(11), 3988-4000(2007)
22. L. Ghellai, H. Hassaine, N. Klouche, A. Khadir, N. Aissaoui, F. Nas, W. ZINGG, Detection of biofilm formation of a collection of fifty strains of Staphylococcus aureus isolated in Algeria at the University, African Journal of Bacteriology Research, 6(1), 1-6(2014)
23. L. Lin, B .D. Ling, X. Z. Li, Distribution of the multidrug efflux pump genes, adeABC, adeDE and adeIJK, and class 1 integron genes in multiple-antimicrobial-resistant clinical isolates of *Acinetobacter baumannii–Acinetobacter calcoaceticus* complex, International journal of antimicrobial agents, 33(1), 27-32(2009)
24. L. J. Kenneth, D. T. Schmittgen Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method, methods, 25, 402-08(2001)
25. T. A. N. V. I. R. Kaur, C. H. A. Y. A. N. I. K. A. Putatunda, A. R. O. M. A. Oberoi, A. S. H. I. S. H. Vyas, G. A. U. R. A. V. Kumar, Prevalence and drug resistance in *Acinetobacter sp.*, isolated from intensive care units patients in Punjab, India, Asian J Pharm Clin Res, 11(14), 88-93(2018)
26. H.Benjamin, B. Reyneke, T. Ndlovu, W. Khan, Genotypic and phenotypic comparison of clinical and environmental Acinetobacter baumannii strains, Microbial pathogenesis, 172, 105749(2022)

27. A. M. I. R. Peymani, S. A. F. A. R. FARAJnIA, M. R. Nahaei, N. Sohrabi, L. A. L. E. H. Abbasi, K. H. A. L. I. L. Ansarin, F. A. T. E. M. E. H. Azhari, Prevalence of class 1 integron among multidrug-resistant *Acinetobacter baumannii* in Tabriz, northwest of Iran, Pol J Microbiol, 61(1),57-60(2012)
28. A. Muhammad, I. A. Alvi, S. U. Rehman, Insight into *Acinetobacter baumannii*: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities, Infection and drug resistance, 1249-60(2018)
29. J. F. Turton, M. E. Ward, N. Woodford, M. E. Kaufmann, R. Pike, D. M. Livermore, T. L. Pitt, The role of IS Aba1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*, FEMS microbiology letters, 258(1), 72-77(2006)
30. H. Kadhom, M. Ali, Epidemiological molecular analysis of *Acinetobacter baumannii* isolates using a multilocus sequencing typing and global lineage, Revis Bionatura, 7, 29(2022)
31. S. M. M. AL-Dahlaki, Molecular detection and gene expression for hcp and *blaOXA-51* genes in *Acinetobacter baumannii* isolated from different clinical sources, MSC Thesis, College of Science, University of Diyala, Diyala, Iraq, 154(2020)
32. M. Jassim, K. I. Mubarak, K. D. Wadi, Study of Antibacterial Activity from Alcoholic Extracts of Torilis arvensis (Huds.) Link on Biofilm formation of *Acinetobacter baumannii* in Diyala Province-Iraq, Academic Science Journal, 1, 60-76(2023)
33. S. M. Kadom, N. A. intidhaar, Detection of bla OXA-51-like and bla VIM Carbapenemase Genes in *Acinetobacter baumannii* isolated from burn patients, International Journal of Pharmaceutical Research, 12, (2020)
34. S. Swaminathan, G. Immanuel, S. Arora, S. Mehta, R. Mittal, A. Mane, S. N. Charugulla, Susceptibility pattern of doxycycline in comparison to azithromycin, cefuroxime and amoxicillin against common isolates: a retrospective study based on diagnostic laboratory data, Journal of The Association of Physicians of India, 68, 59(2020)
35. U. E. I. AL Hadeedy, N. B. Mahdi, I. S. AL Jebory, Isolation and Diagnosis of *Acinetobacter bumanniii* Recently Isolated From Patients in Kirkuk Hospitals and Study

their Antibiotics Resistance, Kirkuk University Journal-Scientific Studies, 14, 155-73(2019)

36. M. A. J. F. M. L. O'conor, D. K. Cadet USMA, C. B. Joseph, Evaluation of Virulence Gene Expression Patterns in *Acinetobacter baumannii* Using Quantitative Real-Time Polymerase Chain Reaction Array, MILITARY MEDICINE, 181, (2017)
37. M. TH. S. AL-Dulaimi, Antimicrobial activity of Colistin combined with some Probiotics against the clinical isolates of *Acinetobacter baumannii* bacteria, MSC. Thesis, College of Science, University of Diyala, (2021)
38. L. Liu, X. Shen, J. Yu, X. Cao, Q. Zhan, Y. Guo, F. Yu, Subinhibitory concentrations of fusidic acid may reduce the virulence of S .aureus by down-regulating sara and saers to reduce biofilm formation and α-toxin expression, Frontiers in Microbiology, 11,25 ,(2020)
39. U. Singkham-In, P. G. Higgins, D. L. Wannigama, P. Hongsing, T. Chatsuwan,.Rescued chlorhexidine activity by resveratrol against carbapenem-resistant *Acinetobacter baumannii* via down-regulation of *AdeB* efflux pump, PLoS One, 15(12), e0243082(2020)
40. M. R. Shakibaie, In Silico Characterization Of Biofilm-Associated Protein (Bap) Identified In A Multi-Drug Resistant *A .baumannii* Clinical Isolate, Journal Of Medical Microbiology And Infectious Diseases, 9, 210- 220(2021)
41. M. A. Narjis, M. S. Mahdi, Isolation and identification of multi-drug resistance *Acinetobacter baumannii* isolated from clinical samples at Baghdad, Iraq, Journal of Applied and Natural Science, 15(2), 663-671(2023)
42. S. Wagner, R. Sommer, S. Hinsberger, C. Lu, R. Hartmann, W. Empting, M. Titz, Novel strategies for the treatment of *Pseudomonas aeruginosa* infections, J. Med .Chem, 59, 5929 − 5969(2016)
43. E. F. Haney, M. J. Trimble, R. E. Hancock, Microtiter Plate Assays To Assess Antibiofilm Activity Against Bacteria, Nature Protocols, 16, 2615-2632(2021)
44. D. S. Auld, P. A. Coassin, N. P. Coussens, P. Hensley, C. Klumpp-Thomas, S. Michael, G. S. Sittampalam, O. J. Trask, B. K. Wagner, J. R. Weidner, Microplate Selection And

Recommended Practices In High-Throughput Screening And Quantitative Biology, Assay Guidance Manual, (2020)

45. Y. Feng, S. Zou, H. Chen, Y. Yu, Z. Ruan, Bacwgstdb 2.0: A One-Stop Repository For Bacterial Whole-Genome Sequence Typing And Source Tracking, Nucleic Acids Research, 49, D644-D650(2021)
46. W. Gamal, C. Brandt, L. D. Sprague, H. Neubauer, M. W. Pletz, WGS based analysis of acquired antimicrobial resistance in human and non-human *Acinetobacter baumannii* isolates from a German perspective, BMC microbiology, 21, 1-13(2021)
47. M. A. Ali, S. S. Mahmood, Role of RND-efflux pump in levofloxacin resistance among Acinetobacter baumannii, Iraqi Journal of Agricultural Sciences, 53,50-542 (2022)
48. C. F. Xu, S. R. Bilya, W. Xu, adeABC efflux gene in *Acinetobacter baumannii*, New microbes and new infections, 30, 100549(2019)
49. B. Choi, M. C. Choudhary, J. Regan, J. A. Sparks, R. F. Padera, X. Qiu, J. Z. Li, Persistence and evolution of SARS-CoV-2 in an immunocompromised host, New England Journal of Medicine, 383(23), 2291-2293(2020)
50. L. Li, J. Yu, X. Shen, X. Cao, Q. Zhan, Y. Guo, F. Yu, Resveratrol enhances the antimicrobial effect of polymyxin B on *Klebsiella pneumoniae* and *Escherichia coli* isolates with polymyxin B resistance, BMC microbiology, 20, 1-8(2020)
51. C. B. Landersdorfer, N. S. Ly, H. Xu, B.T. Tsuji, J. B. Bulitta, Quantifying subpopulation synergy for antibiotic combinations via mechanism-based modeling and a sequential dosing design, Antimicrobial agents and chemotherapy, 57(5), 2343-2351(2013)
52. E. J. Yoon, P. Courvalin, C. Grillot-Courvalin, RND-type efflux pumps in multidrug-resistant clinical isolates of *Acinetobacter baumannii:* major role for AdeABC overexpression and AdeRS mutations, Antimicrobial agentsand chemotherapy, 57(7), 2989–2995(2013)
53. A. J. Seukep, H. G. Mbuntcha, V. Kuete, Y. Chu, E. Fan, M. Q. Guo, What Approaches to Thwart Bacterial Efflux Pumps-Mediated Resistance, Antibiotics, 11(10), 1287(2022)

54. A. Duartea, C. Ana Alves AC, S. Ferreira, F. Silva, FC. Dominguesa, Resveratrol inclusion complexes: Antibacterial and anti-biofilm activity against Campylobacter spp. and Arcobacter butzleri, Food Res Int, 77(2),244 –250(2015)
55. L. Kursvietiene, I. Staneviciene, A. Mongirdiene, J. Bernatoniene, Multiplicity of effects and health benefits of resveratrol, Medicina (Kaunas), 52,148–155(2016)
56. P. Verma, M. Tiwari, V. Tiwari, Strategies to combat bacterial antimicrobial resistance: a focus on mechanism of the efflux pumps inhibitors, SN Comprehensive Clinical Medicine, 3, 510-527(2021)