

DOI: <http://dx.doi.org/10.21123/bsj.2022.19.2.0255>

## Colistin as A Good Monotherapy to Restrain the Pathogenicity of *Acinetobacter baumannii* In vivo and In vitro

Nagham Saad Mohammed

Halah H. Al-Haideri\*

Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq

\*Corresponding author: [naghamsaadi94@yahoo.com](mailto:naghamsaadi94@yahoo.com), [halahm\\_bio@scw.uobaghdad.edu.iq](mailto:halahm_bio@scw.uobaghdad.edu.iq) \*

\*ORCID ID: <https://orcid.org/0000-0002-3611-2456>, <https://orcid.org/0000-0001-7495-0162> \*

Received 23/10/2020, Accepted 4/1/2021, Published Online First 20/9/2021, Published 1/4/2022



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

### Abstract:

*Acinetobacter baumannii* (*A. baumannii*) is a major opportunistic nosocomial pathogen, mostly resistant to several groups of antibiotics. Colistin is now used as a last-line treatment for isolates that are highly resistant. The purpose of this study is to identify the importance of LptD; which is involved in the translocation of LPS from the inner membrane to the outer membrane in compartment with LptA and LptC of *A. baumannii* and its indispensable role as a virulence factor, and the efficiency of colistin as a monotherapy. In the current research, two isolates of *A. baumannii* were used, the local isolate HHR1 isolated from urine sample and the global strain ATCC 17904, and three antibiotics (colistin, rifampicin and Fosfomycin) were used as a monotherapy and synergic therapy in vivo and ex vivo. The results demonstrated that *A. baumannii* HHR1 was more resistant to Antimicrobial peptides (AMPs) than the standard strains. The effect of AMPs on *A. baumannii* was increased by increasing the concentration and the time of incubation, and also AMPs were shown to be lethal on *A. baumannii* growth spatially at high concentration ( $2 \mu\text{g ml}^{-1}$ ) in monotherapy and ( $1.5, 2 \mu\text{g ml}^{-1}$ ) in synergic. The susceptibility of isolates towards antibiotics was variable, where colistin exerts significant growth had defect as a monotherapy and in combination with others. The results showed that the expression of *lptD*, *lptA* genes of *A. baumannii* HHR1 were higher than of the same genes in *A. baumannii* ATCC 17904 in the presence of  $2 \mu\text{g ml}^{-1}$  colistin, while *lptE* gene of the *A. baumannii* ATCC 17904 showed an upregulation pattern than in *A. baumannii* HHR1. Furthermore, colistin influences the adhesion ability of *A. baumannii* on epithelial cells (A-549 lung cancer cell) by reducing the number of cells, and thus could colistin be a good candidate for *A. baumannii* treatment.

**Keywords:** *Acinetobacter baumannii*, AMPs, lipopolysaccharide, q-PCR.

### Introduction:

*Acinetobacter* spp. is a non-fastidious Gram-negative bacterium, which belongs to gammaproteobacteria, aerobic and non-motile, non-fermenting. In addition to the high GC content, it is reported to be negative for oxidase and positive for catalysis<sup>1</sup>. The genus *Acinetobacter* contains 31 well-known species that occur chiefly in nature and are mainly concerned with nosocomial infection, urinary tract infection, skin and other soft tissues, and in the blood stream<sup>2</sup>

The prevalence of multidrug-resistant pathogens has gradually increased and become a source for serious concern for both nosocomial and community-acquired infections. Presumably, *A. baumannii* is sensitive to most antibiotics, at present, the pathogen performs to show broad

resistance to most first-line antibiotics<sup>3</sup>. Antimicrobial peptides exhibit wide-spectrum and intense antimicrobial activity against bacteria, fungi and viruses<sup>4,5</sup>. Amongst the main constituents of the innate immune system are AMPs which were identified in a variety of species, such as bacteria, fungi, animals and plants, and the majority of cationic AMPs that play an important role in antimicrobials<sup>6</sup>. Colistin is an AMP that works on LPS. The LPS is a macromolecule containing the hydrophobic group (lipid group), and a hydrophilic group (the primary oligosaccharide), and O antigen. LPS is biogenesis occurred in the inner membrane (IM) and translocate to the outer membrane (OM) by the LPS vector comprising seven subunits, LptA, LptB, LptC, LptD, LptE, LptF, and LptG<sup>7</sup>.

Therefore, transporting the LPS across the water-filled ocean and the LptD barrel is quite challenging. However, it is suggested that the outermost LptD / E membranous proteins are responsible for the formation of the periplasmic segment with LptA and LptC to translocate LPS from the IM to the OM. Colistin is an AMP that binds to the outer cell membrane of Gram-negative bacteria. Its mechanism starts on the binding to the lipopolysaccharides in the outer membrane, and then an electrostatic reaction takes place between the alpha and beta-diaminobutyric acid of colistin and the phosphate groups in the lipid region A of the lipopolysaccharide (LPS). The divalent cations (Ca<sup>2+</sup> and Mg<sup>2+</sup>) was competitively replaced from the phosphate groups of membrane lipids <sup>8,9</sup>. As a result, the distraction of LPS may increase the permeability of the OM and intracellular contents leakage, and cell death <sup>10</sup>. Unfortunately, the development of colistin-resistant isolates has been commonly recorded over the last few decades <sup>9,11</sup>, which has increased the incorrect usage of this agent, particularly as monotherapeutic treatment, and thus could be the cause of this problem <sup>12</sup>. Unfortunately, the rapid development of colistin resistance among clinical isolates of *A. baumannii*, as a result of misuse of colistin has led to threatening the future life with increasing usage of this antibiotic. Using rifampin and fosfomycin as a monotherapeutic agent didn't end up with complete killing for both isolates, whereas, using synergism combination of colistin-rifampin, and colistin-fosfomycin was remarkably inhibited the viability of both isolates <sup>13,14</sup>.

## Materia and Methods:

### Bacterial Sample Preparation

*A. baumannii* ATCC 17904 and local isolates HHR1 with the accession no. (MH685112.1) were used in this study. The standard isolate was purchased and the HHR 1 was isolated from patient urine. The isolates were routinely cultured on Nutrient Agar (NA) and MacConkey Agar (MAC) media under aerobic condition at 37°C for 24 hours. For growth curve, bacterial isolates were overnight cultured and in Mueller Hinton (MH) broth under the conditions above with genital shaking at 180 rpm. Bacterial growth curve was performed either under normal conditions as a control, or with the presence of AMP. In both cases, the growth of bacterial isolate was monitored by the measurement of OD<sub>600</sub> against the relevant media as a control (blank). The growth was observed every hour started from OD<sub>600</sub>~0.1 to approximately OD<sub>600</sub> 1.5.

### Genomic DNA Extraction and Polymerase Chain Reaction

#### A- DNA Extraction

DNA extraction from isolates was done by using wizard kit's protocol (Promega) according to the manufacturer's instructions. DNA was stored at -20°C for further use. The genes of interest of two *A. baumannii* isolates amplified by PCR using primers listed in Table 1 were purchased from Alpha DNA/Canada. The purity and concentration of the DNA were estimated by nanodrop device. The concentration of each primer was adjusted to 10 μM using dH<sub>2</sub>O. Specific primers that were needed for amplifying fragment of LptD-F/LptD-R, LptE-F/LptE-R, LptA-F/LptA-R, and rplB-F/rplB-R were listed in Table 1.

**Table 1. Primers used in this study**

Primer name	Primer sequence	Reference	Gene product size
LptD-F	5`GAGCTCGGTACCCGGGGATCCTCTAGAGTCGCGCATCCATAAG CATATAC-3`	Designed in this study	720 bp
LptD-R	5`AAAGCTTATCGATGATAAGCTGTCAAACATGCTAACTGTACTCG GCCTTG-3`		
LptE-F	5`-CCTCGTCGTCAGCTTCTAAAT-3`		100 bp
LptE-R	5`-ACTACGAGCTGCGGTAAAG-3`		
LptA-F	5`-CCGTCTGACCGTAATCAACAA-3`		100 bp
LptA-R	5`-CCCTGCTCAATCACGACATTA-3`		
rplB-F	5`GTAGAGCGTATTGAATACGATCCTAACC-3`	<sup>15</sup>	330 bp
rplB-R	5`- CACCACCACCATGCGGGTGATC-3`		

#### B- Preparation of PCR Mixture

All reactions were performed with GoTaq Green Master mix (Promega). 25μl PCR reaction was used that contained 12.5μl Mastermix, 2.5μl of each primer, 2μl of genomic DNA as a template, and

sterilized water to make final volume up to 25μl. Amplification program was conducted as follows: initial denaturation step at 95°C for 5min, followed by 35 cycles at 95°C 30sec for denaturation, annealing at 66°C for *lptD* and 60°C for *lptE*, and

*lptA* 30 sec, the extension was at 72°C 30sec, final extension at 72°C for 7min, and holding at 4°C. The PCR product was examined on agarose gel to confirm that there was a specific product with the desired size. Table (2), conditions of PCR for *lptD*, *lptE*, and *lptA*.

#### Effect of AMP on *A. baumannii* (In vitro) Colony Counting Assay

In order to determine the effect of AMPs on the viability of *A. baumannii* isolates, a fresh culture of isolates in MH broth was incubated as previously mentioned, the growth was monitored and the OD<sub>600</sub> was adjusted to 0.5 prior AMPs addition. A 96 microtiter wells plate was used to measure the activity of different AMPs toward *A. baumannii* isolates. Serial dilution was conducted with bacterial cells of  $5.8 \times 10^8$  c.f.u in the presence of several concentrations of AMPs all plates were incubated under aerobic conditions, and 20 µl of antibacterial culture was serially diluted in MH broth. A10 µl of all dilutions was spotted on MH agar for CFU calculation after 24 hr incubation<sup>16</sup>. All assays were performed in triplicate.

#### RNA Extraction and Quantitative PCR Analysis (qPCR)

RNA of *A. baumannii* isolates was extracted by using TRIzol™ Reagent, according to the protocol described by the manufacture. RNA was extracted from fresh bacteria culture, and the concentration of extracted RNA was detected by using Quantus Fluorometer. Quantitative PCR analysis (qPCR) of *lptD*, *lptE*, *lptA* and *rplB* was employed by using GoTaqR 1-Step RT-qPCR System, this system was a reagent system for quantitative analysis of RNA using a one-step reverse transcription-quantitative PCR (RT-qPCR) protocol. The primer sequences for genes are listed in Table 1. The gene *rplB* was used as a housekeeping gene for the quantification of *lptD*, *lptE* and *lptA*. The optimization program was done as listed in Table 2. Cycles of reverse transcription were at 37°C for 15 minutes, initial denaturation/ RT inactivation was at 95°C for 10 minutes. Then 40 cycles of denaturation at 95°C for 15 seconds was done, annealing was done at 66°C for *lptD*, 60 °C for *lptE*, *lptA* and 50 for *rplB* for 30 seconds, and extension was performed at 72°C for 30 seconds in each cycle. The Quantitative PCR analysis (qPCR) of samples was done by using Mic qPCR Cycler (Bio Molecular System, Australia).

**Table 2. qPCR optimization program for the genes (*lptD*, *lptE*, *rplB*, and *lptA*).**

Optimization	Temp. °C	Time	No. of cycles
Reverse Transcription Initial	37	15 min	1 cycle
denaturation/ RT inactivation	95	10 min.	
Denaturation	95	20 sec.	
Annealing	<i>lptE</i>	60	50 cycle
	<i>lptA</i>		
	<i>lptD</i>	66	
Extension	<i>rplB</i>	50	20 sec.
		72	

#### Colistin Activity on Cell Line (ex vivo) Cell Cultures

Lung cancer A-549 Cell lines were attained from the Iraqi biotech Cell Bank Unit and these cells were preserved in RPMI-1640 supplemented with 10% Fetal bovine, 100 units mmL<sup>-1</sup> penicillin, and 100 µg ml<sup>-1</sup> streptomycin. Cells were equipped with Trypsin-EDTA and reseeded at 50% confluence twice a week, and incubated at 37°C<sup>17</sup>.

#### Bacterial Adherence Assay

Lung cancer cell was planted in 24 well tissue culture plates at density  $1 \times 10^5$ . After 24 hr, cells were infected with both strains of *A. baumannii* ATCC 17904 and HHR1 at a ratio of 50:1 (MOI of 50). The cells infected with bacteria in the presence and absence of (colistin) at concentration MIC 2 µg ml<sup>-1</sup> were incubated in a 5% CO<sub>2</sub> at 37°C for 8 hr. The cells were washed 3 times with PBS, then fixed with 4% PFA for 30 min, washed as above and stained with Giemsa's stain<sup>17,18</sup>.

#### Results:

##### Gene Detection

In order to detect the genes involved in LPS biogenesis, DNA was extracted from both strains, and genes of interest *lptD*, *lptE*, *lptA* were PCR amplified as described previously (Fig. 1). The PCR products were electrophoresed on 1 % agarose gel, 100 V for 60 min. The PCR products for all samples were visualized and appeared as a clear band with corresponding size of 720 bp for *lptD* and 100bp for *lptE*, *lptA* respectively (Fig. 2).

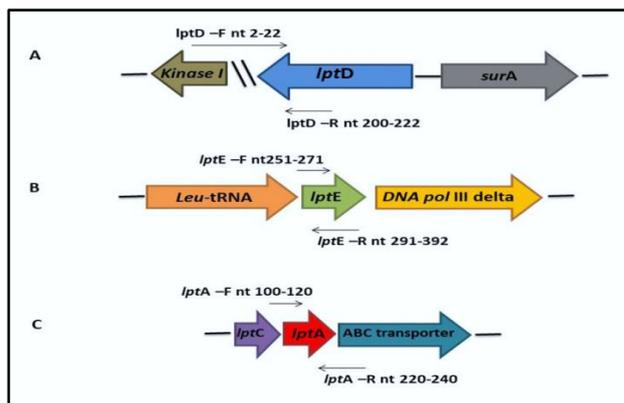


Figure 1. Gene map of Detected of *lptD*, *lptE* and *lptA* genes in *A. baumannii*.

The genes of interest were PCR amplified using the designed primer pairs (A) *lptD* gene organization map with the upstream and downstream genes; (B) gene organization map of *lptE* refers to its location and amplified base pairs (C) *lptA* with upstream and downstream genes.

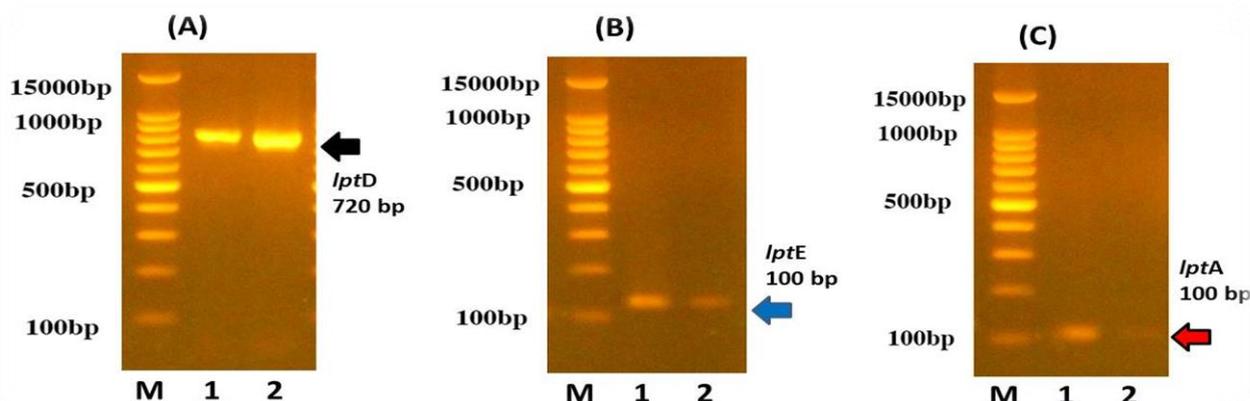


Figure 2. Agarose gel of electrophoresis of *lptD*, *lptE*, *lptA* genes in *A. baumannii*. The PCR products of genes of interest in *A. baumannii* isolates were loaded on 1% agarose gel, 100 v for 60 min . (A) The 720 bp products refer to *lptD* gene and indicated by black arrow. (B) *lptE* gene with the relative size of 100 bp as indicated by blue arrow refer ;(C) the red arrow refers to *lptA* with the size of 100 bp. In all cases, lane 1 represents the standard strains ATCC 17904, and lane 2 refers to the local isolate HHR.

## Bacterial Growth Curve

### Effect of AMP on *A. baumannii* Growth

In colistin, the growth of both isolates reached up to OD<sub>600</sub> of 0.5, while the control ended up to OD<sub>600</sub> of 1.0 in *A. baumannii* ATCC, 0.9 in *A. baumannii* HHR1 after 12 hr incubation. Most notably, the growth of *A. baumannii* HHR1 was monitored at OD<sub>600</sub> 0.19, 0.130, 0.09 after 12 hrs incubation for 1, 1.5, and 2 μg ml<sup>-1</sup> respectively, while the growth of ATCC at OD<sub>600</sub> 0.1, 0.12, 0.08 for 1, 1.5, and 2 μg ml<sup>-1</sup> respectively after the same period (Fig. 3 A, B).

In the presence of fosfomycin, and the growth of the three concentrations after 12 hours of the incubation period, the control reached up to OD<sub>600</sub> of 0.89 in *A. baumannii* ATCC. In contrast, the growth of both isolates ended up at OD<sub>600</sub> 0.69 and 0.68, 0.61, in *A. baumannii* ATCC and 0.79, 0.77, 0.72 in *A. baumannii* HHR1 respectively. (Fig. 3 C, D). On the other hand, with rifampicin, after the same incubation period, the control reached up to OD<sub>600</sub> of 0.96 in *A. baumannii* ATCC and 0.970 in *A. baumannii* HHR1, whereas when the growth of

both isolates after 12 hours incubation, the control reached up to OD<sub>600</sub> of 0.96 in *A. baumannii* ATCC and 0.970 in *A. baumannii* HHR1, and the growth of both isolates were reached in the presence of 1, 1.5, and 2 μg ml<sup>-1</sup> up to OD<sub>600</sub> 0.805 and 0.694, 0.634, in *A. baumannii* ATCC and 0.77, 0.71, 0.69 in *A. baumannii* HHR1 respectively (Fig. 3 E, F).

In the combination of serial concentrations of colistin-fosfomycin, colistin rifampicin (Fig. 4 A, B, C, D), the growth of both isolates in the first hour reached up to OD<sub>600</sub> of 0.52, 0.443, 0.440 in *A. baumannii* ATCC, and 0.533, 0.439, 0.429 in *A. baumannii* HHR1 in comparison to the control that ended up to OD<sub>600</sub> of 1.05 after 12 hrs incubation.

### Cytotoxic Effect of AMPs on *A. baumannii*

There is no significant difference between the first three concentrations of colistin, while, 2 μg ml<sup>-1</sup> exerts significant differences (\*\*P<0.0002) between both strains of *A. baumannii* (Fig. 5 A). In fosfomycin there is no significant difference between the first three concentrations while, the

high concentration ( $35 \mu\text{g ml}^{-1}$ ) significantly differs ( $****P<0.0001$ ) between both strains of *A.baumannii* (Fig. 5 B). Similarly, the fosfomycin-colistin combination, no significant difference between the first three concentrations against *A. baumannii* strains, while,  $2 \mu\text{g ml}^{-1}$  shows significant difference ( $*P<0.0110$ ) between both strains of *A.baumannii* (Fig. 5C).

In addition, no significant difference has been observed between the first three concentrations of

Rifampicin, while,  $2 \mu\text{g ml}^{-1}$  shows significant differences ( $****P<0.0001$ ) between both strains of *A.baumannii* (Fig. 5D), and there is no significant difference between the first two concentrations of Rifampicin-colistin combination used against *A. baumannii* strains, while,  $1.5, 2 \mu\text{g ml}^{-1}$  is significant different ( $**P<0.0011$ ), ( $*P<0.0129$ ) between both strains of *A.baumannii* (Fig. 5E).

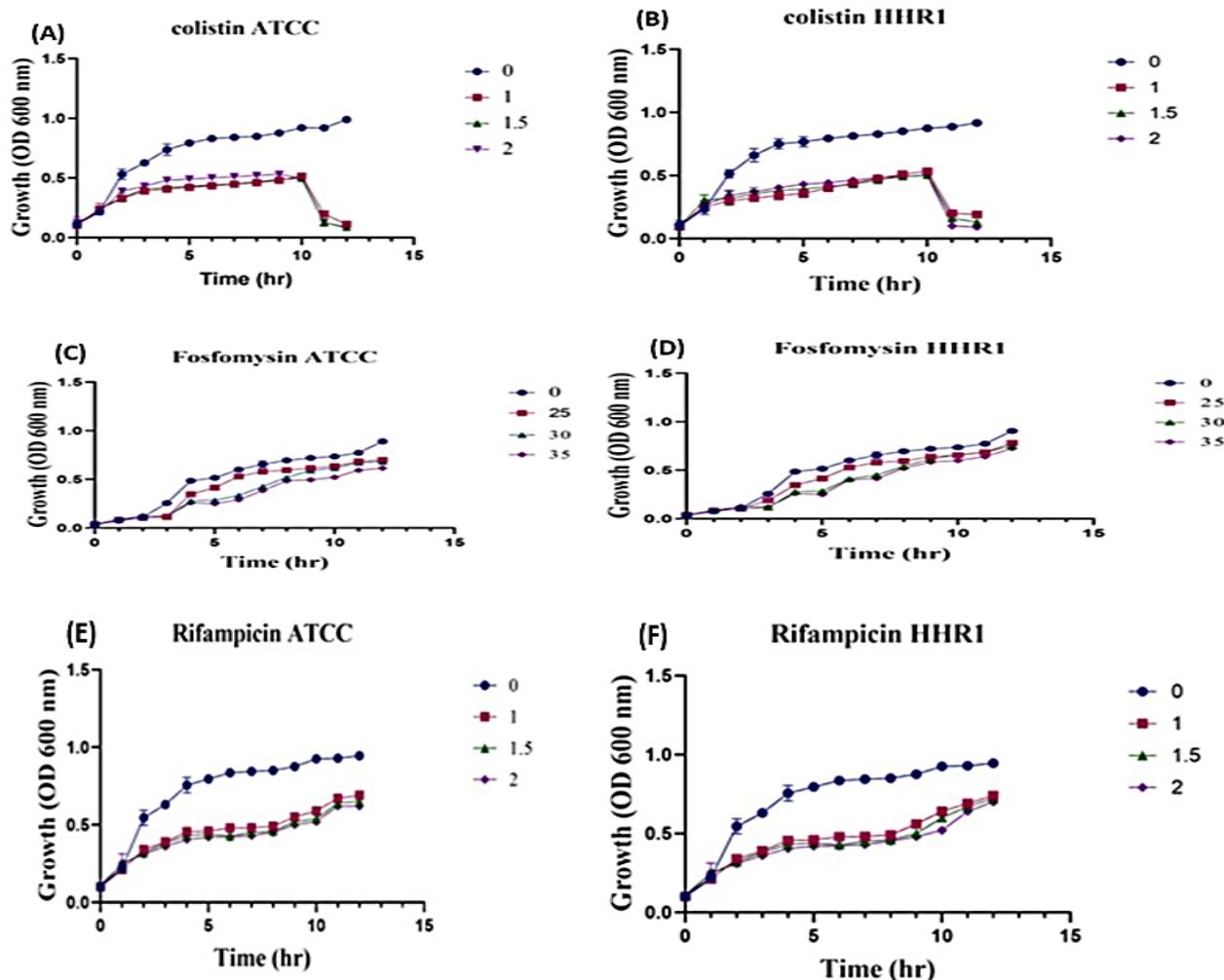


Figure 3. Growth of *A. baumannii* strains in the presence of AMPs: 100 ml of *A. baumannii* ATCC 17904 and HHR1 was taken. Cultures were grown overnight and the growth was prepared by inoculating each culture to an  $\text{OD}_{600}$  of 0.1, before being incubated under aerobic conditions. Three concentrations of AMP were added to each culture after 1 hour incubation period, otherwise, the cultures were left without AMP addition determined as control. In both cases, the growth was monitored every hour up to 12 hr. All assays were performed in three biological replicates.

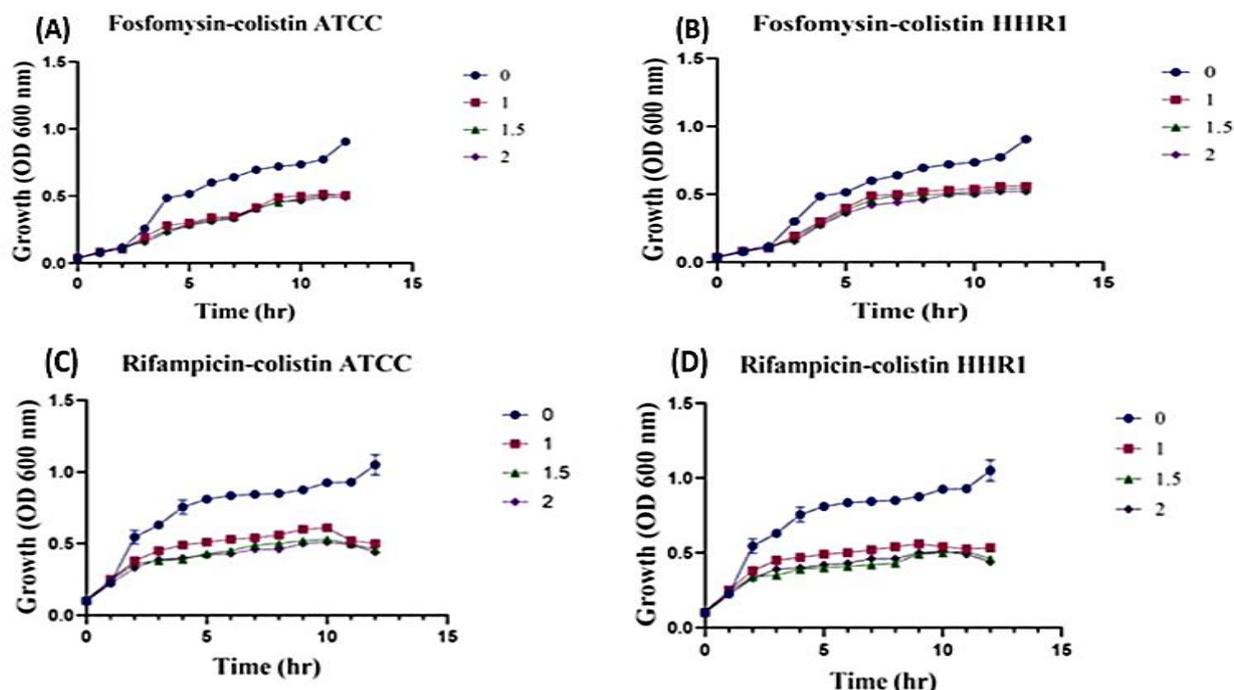


Figure 4. Growth of *A. baumannii* strains in the presence of combination of AMPs: 100 ml of *A. baumannii* ATCC 17904 and HHR1 cultures were grown overnight and the growth was prepared by inoculating each culture to an OD<sub>600</sub> of 0.1, before being incubated under aerobic conditions. Three concentrations of AMP were added to each culture after 1 hour incubation period, otherwise, the cultures were left without AMP addition determined as control. In both cases, the growth was monitored every hour up to 12 hr. All assays were done by three biological replicates.

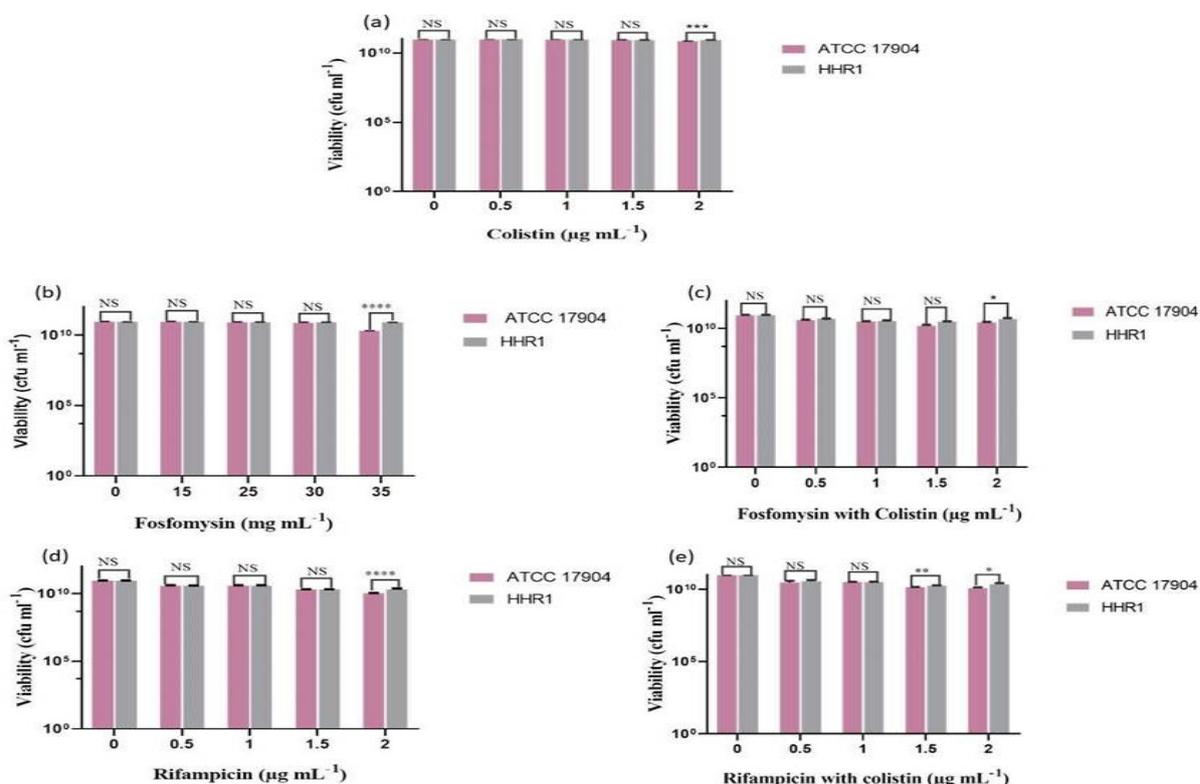


Figure 5. Antimicrobial susceptibility on the viability of *A. baumannii* strains. *A. baumannii* cultures were incubated with different concentrations of AMP (0.5, 1, 1.5, 2 μg mL<sup>-1</sup>) for 1 hr at 37°C, before being plated on MH agar plates for cfu determination. Error bars represent the standard error from the mean of three independent biological replicates.

### Quantitative PCR Analysis (qPCR)

In this study, the mRNA levels of *lptD*, *lptA* were decreased in both strains at  $2 \mu\text{g ml}^{-1}$  concentrations of colistin, but in HHR1 the levels were higher than in standard strain. (Figs. 6, 7) (55). On the other hand, the mRNA levels of *lptE* were strongly increased in HHR1 in the presence of colistin compared with ATCC. (Fig.7).

### Effect of Colistin on Adherence of *A. baumannii* on A-549 Cell

The results showed that *A. baumannii* invaded and adhered to the A-549 (Fig. 8).

### The Half Inhibitory Concentration (IC<sub>50</sub>) of Colistin on *A. baumannii*

The IC<sub>50</sub> values of colistin can be calculated from the viability results to determine the inhibitory concentration of the *A. baumannii* and cytotoxicity concentration on *A. baumannii* (Fig. 9).

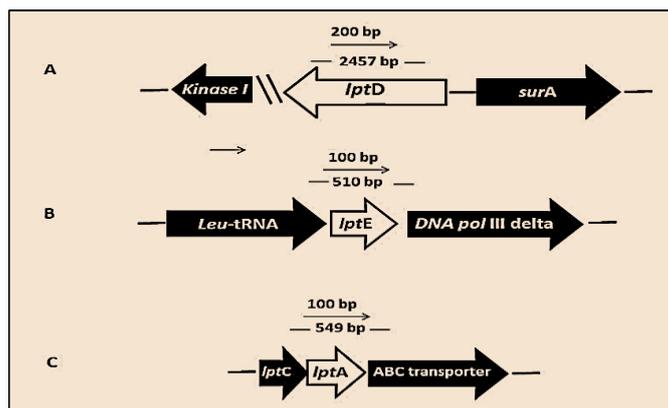


Figure 6. Detection of *lptD*, *lptE* and *lptA* genes in *A. baumannii*. (A) *lptD* gene organization map. (B) *lptE* gene organization map. (C) *lptA* The location of *lptD*, *lptE* and *lptA* genes were determined and specific primers were designed

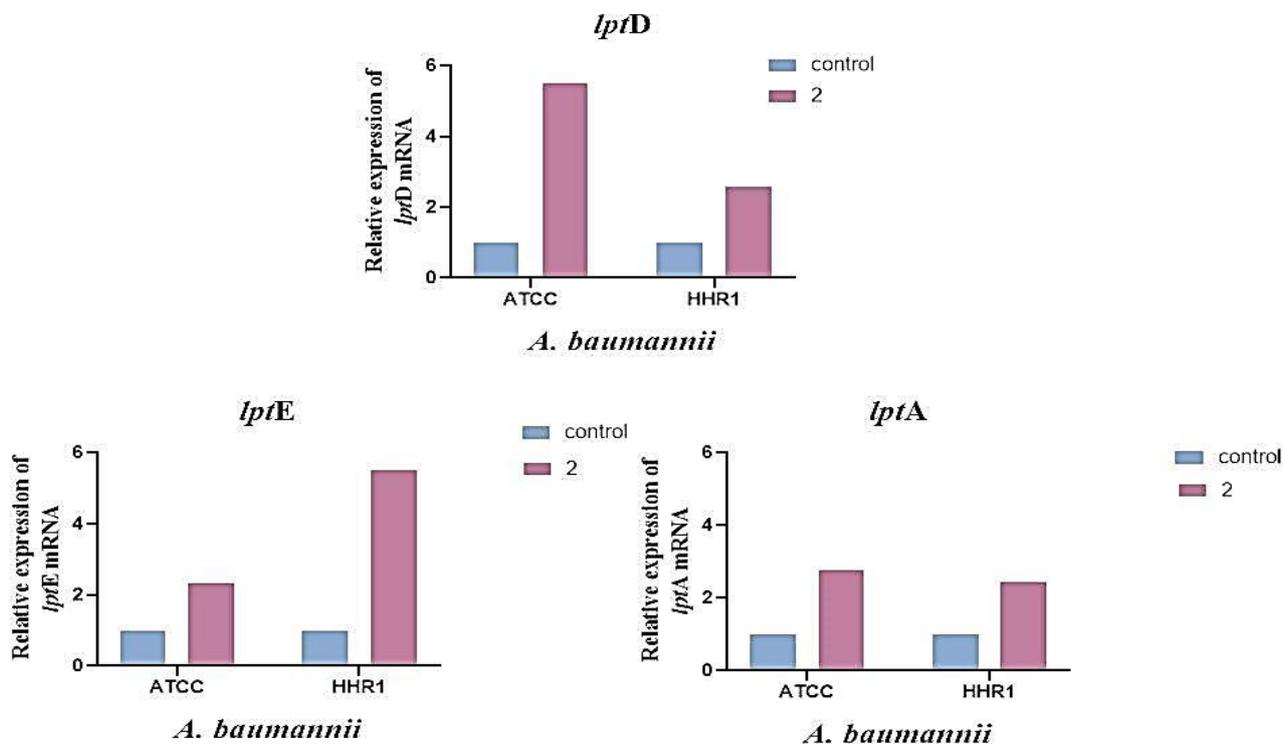
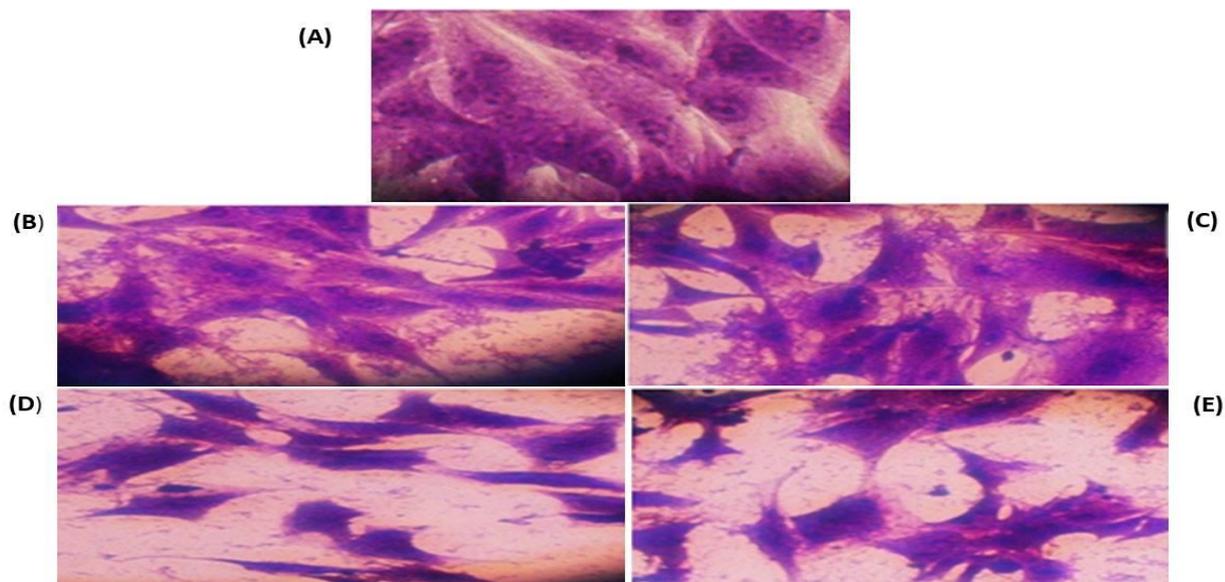
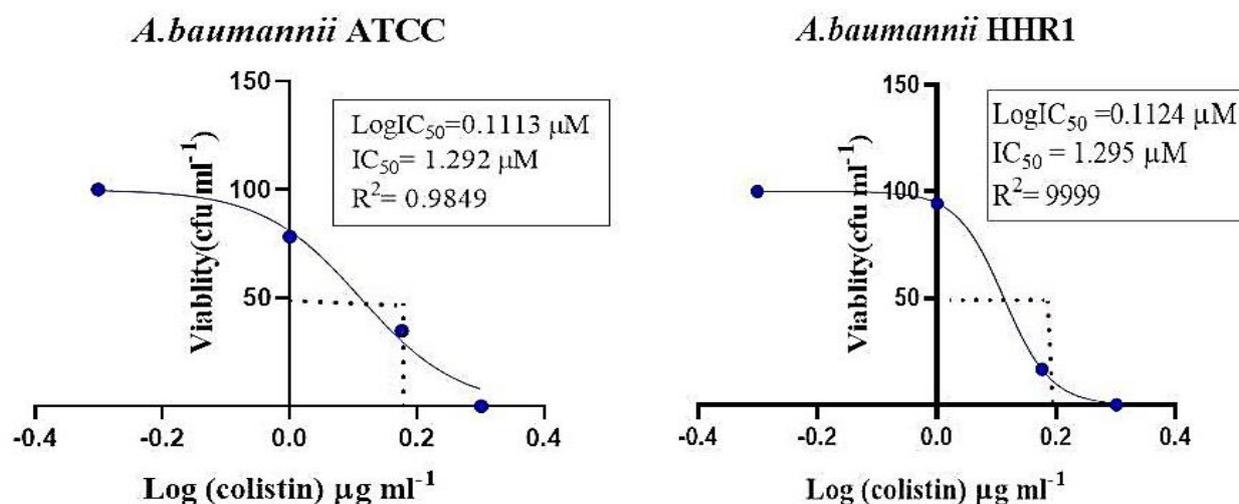


Figure 7. Effect of colistin ( $2 \mu\text{g ml}^{-1}$ ) on the expression levels of several genes that participate in antibiotic resistance in *A. baumannii* ATCC 17904 & HHR1.



**Figure 8. Effect of colistin on adherence of *A. baumannii* on lung cancer cells.** A-549 cells stained with Giemsa stain then examined under inverted microscope with 40X magnifications. (A) Normal A-549 cell. (B) A-549 cell infected with *A. baumannii* ATCC 17904. (C) A-549 cell infected with *A. baumannii* HHR1. (D) A-549 cell infected ATCC 17904 treated with 2 ( $\mu\text{g ml}^{-1}$ ) of colistin. (E) A-549 cell infected with HHR1 treated with 2 ( $\mu\text{g ml}^{-1}$ ) of colistin.



**Figure 9. IC50 of colistin on both isolates of *A. baumannii* after 24 hr.**

## Discussion:

### Bacterial Growth Curve

#### Effect of AMP on *A. baumannii* Growth

*A. baumannii* isolates exhibit a variable response to AMP, colistin is selected to determine its effect on the viability of *A. baumannii* isolates as monotherapy and synergic with other antibiotics (fosfomycin, rifampicin). Colistin is one of the most AMPs typically used due to its effectiveness, there is less variability in pharmacokinetic, and no regulation is required in the case of renal failure. The results exhibit that colistin affected the growth of *A. baumannii* as shown in Fig. 3 A, B.

The results revealed that different concentrations of colistin remarkably affected *A. baumannii* growth in the early hours. Most notably, the growth of *A. baumannii* HHR1 and *A. baumannii* has been decreased in the same period.

*A. baumannii* can alter AMP resistance by completely losing the primary binding site, the lipid component A of the lipopolysaccharide, which is crucial for the survival of Gram-negative bacteria. Previous studies, revealed that the mutations in (*lpxA*, *lpxC*, and *lpxD*); the essential genes biosynthesis pathway of LPS in 13 individual derivatives of colistin-resistant *A. baumannii* ATCC 19606, increased the resistance patterns of isolates

<sup>19</sup>. *A. baumannii* exerts variable resistance to colistin, as an apparent colistin susceptible strain harbored a small proportion of colistin-resistant cells <sup>20</sup>. Under selective stress conditions in vitro and in vivo, heteroresistant strains of *A. baumannii* can rapidly lead to the development of strains with a high level of resistance to colistin <sup>21,22</sup>. The main resistance mechanisms of colistin are the modulation of LPS by adding phosphoethanolamine to lipid A; so, this modification decreases the affinity of colistin with the surface of bacterial cells by decreasing the negative charge of the OM, and the mutation in genes for LPS synthesis, also this mechanism differs between strains of *A. baumannii* <sup>19</sup>. Several studies have speculated that in vitro antimicrobial activity increased further when the exposure time simulates over 24 hours. The ability of some antimicrobials to completely kill or obliterate bacteria, enhances the activity of other AMPs to inhibit bacteria in synergism way <sup>13,23</sup>. The killing activities of some AMPs as a monotherapy may produce rapid bacterial resistance with high concentration which may result in severe toxicity. Several studies revealed that bacterial resistance is related with monotherapeutic agent, e.g; rifampin displayed both in vitro and in vivo bactericidal activities against MDR *A. baumannii* in pneumonia experimental model, while, rifampin-resistant mutants exert rapid and short performance after the initiation of treatment <sup>24,25</sup>. Colistin is a last resort treatment, a well-known AMP from the polymyxin family.

Although, colistin is efficient against MDR- *A. baumannii* isolates, the efficacy and consequence toxicity of colistin as a monotherapy which increased the potential resistance should be taken into consideration <sup>26</sup>. Colistin employs a rapid bactericidal effect at high and low concentrations, by affecting the OM and increasing the permeability of Gram-negative bacteria, which facilitates the entry of excluded compounds <sup>27</sup>. When fosfomycin is added to *A. baumannii* isolates, the growth is similar to the control of the three concentrations, and the growth increases when the incubation period is increased for both isolates after 12 hours of the incubation process in which the control of *A. baumannii* ATCC is slightly higher than all treatment, then after 12 hours of the incubation process both isolates decreased, although the results are similar to *A. baumannii* ATCC and HHR1. In ATCC the growth is slightly less than the local (Fig. 3 C, D). On the other hand, rifampicin is added to *A. baumannii* isolates, the growth is similar to the control of the three concentrations, and the growth increases when the incubation period is increased for both isolates after 12 hours of the incubation

process in which the control of *A. baumannii* ATCC is slightly higher than all treatment. Later, after 12 hours of the incubation process both isolates decreased, although the results are similar to *A. baumannii* ATCC and HHR1. In ATCC the growth is slightly less than the local (Fig. 3 E, F).

Furthermore, increasing resistant ability of *A. baumannii* strains toward general antibiotics, carbapenems in particular rose the potential using of colistin for infection treatment. Some of in vivo studies reported the individual using of colistin or in combination with rifampicin infections treatment. In the combination of serial concentrations of colistin-fosfomycin, colistin rifampicin (Fig. 4 A, B, C, D), the growth is slightly increased in the first hour where both isolates in comparison to the control after 12 hrs incubation.

Rifampicin is an antibiotic that is often used as synergism with other antibiotics; however, using rifampicin in combination depends on multiple conditions such as the rifampicin MIC or the methods used, for example <sup>28,29</sup>. We have cast the light on the studies that tested the synergism effects of rifampin and colistin against both strains of *A. baumannii* in vitro. This study aims to examine the activity of rifampicin, colistin and fosfomycin as monotherapy and with a synergistic effect on both strains of *A. baumannii*. The range of MIC for rifampin and colistin is 1.5  $\mu\text{g ml}^{-1}$  and 2  $\mu\text{g ml}^{-1}$ .

#### Cytotoxic Effect of AMPs on *A. baumannii*

The viability calculation of *A. baumannii* strains after exposure to different concentrations of AMP (0.5, 1, 1.5, and 2  $\mu\text{g ml}^{-1}$  for colistin and rifampicin, and 15, 25, 30, and 35  $\text{mg ml}^{-1}$  for fosfomycin) was measured using Colony Forming Unit (CFU). *A. baumannii* treated with AMP exhibit a remarkable decrease in viability percentage compared with the control (bacteria lacking AMP).

These results improved the efficiency of AMP in decreasing the viability percentage of the *A. baumannii* as shown in Fig 5, they also revealed that the local isolate HHR1 exhibits high resistance to AMP than the standard strain, and also shows that viability percentage decreases by increasing AMP concentration, the former is approachable because of the rapid effectiveness, low cost, simple dosage requirement, and short time for serum concentration, the variability in pharmacokinetics is less, and no modification is required for renal failure. However, in our patient, using of colistin is required due to the need to covering of pulmonary nidus and the possibility of inhalation <sup>30,31</sup>. The results show that there is no significant difference between the first three concentrations of colistin used against *A. baumannii* strains, while, 2

$\mu\text{g ml}^{-1}$  exerts significant between both strains of *A.baumannii* (Fig. 5 A). Furthermore, fosfomycin is a broad-spectrum agent that is mainly used in combination therapy because it reduces the MIC of MDR strains and using as a monotherapy encourages the development of resistance<sup>32</sup>. The results show that there is no significant difference between the first three concentrations of fosfomycin used against *A. baumannii* strains, while, the high concentration ( $35 \text{ mg ml}^{-1}$ ) is significantly differ between both strains of *A.baumannii* (Fig. 5 B).

The synergy between colistin and fosfomycin was controversial. Previous studies revealed that the combination of colistin-fosfomycin showed a synergistic effect in half of their examined isolates. Few studies demonstrated the colistin-fosfomycin combination, the formulation of these combining AMPs was revealed to demonstrate the cons of using colistin-fosfomycin against MDR *A. baumannii*. The opinion was different from one scientist to another, then the results show that there is no significant difference between the first three concentrations of fosfomycin-colistin combination used against *A. baumannii* strains, while,  $2 \mu\text{g ml}^{-1}$  shows significant difference between both strains of *A.baumannii* (Fig. 5C).

Rifampicin is used as a patient antibiotic that, despite its effectiveness as an individual drug, exhibits a potential activity when mixed with other antibiotics, colistin in particular, by increasing of the bacterial membrane permeability and increasing the effectiveness of the treatment<sup>33</sup>. A new therapy proposal for patients with complex UTIs caused by MDR pathogens could be on the horizon. The addition of rifampin to all colistin regimens resulted in increasing bacterial killing and regrowth dilution similarly to monotherapy such that after 24 to 72 hours at least, no viable bacteria were detected. Moreover, increasing bacterial killing was particularly obvious for formulations containing colistin  $0.5$  or  $2 \text{ mg l}^{-1}$  systems, with synergism produced on all occasions from 24 h onwards. For example, while no viable bacteria were detected using either combination regimens of  $0.5$  and  $2 \mu\text{g ml}^{-1}$  of colistin at 24 hours, significant bacterial killing occurred at  $1.5$  and  $2 \mu\text{g ml}^{-1}$  of the colistin monotherapy regimen the results show that there is no significant difference between the first three concentrations of Rifampicin used against *A. baumannii* strains, while,  $2 \mu\text{g ml}^{-1}$  gave significant differences between both strains of *A.baumannii* (Fig. 5D), and there is no significant difference between the first two concentrations of Rifampicin-colistin combination used against *A. baumannii* strains, while,  $1.5, 2 \mu\text{g ml}^{-1}$  showed significant differences between both strains of *A.baumannii*

(Fig. 5E). The synergistic treatment against colistin-susceptible isolate completely suppresses the development of colistin-resistant bacterial populations at both inocula. Previous studies showed that the combination of AMPs with  $2$  or  $5 \text{ mg l}^{-1}$  colistin regimen decreased the occurrence of colistin-resistant isolates under the limit of detection (i.e.,  $20 \text{ CFU ml}^{-1}$ ) at low inoculum<sup>34</sup>.

This result referring to the use of colistin-rifampin in combination treatment resulted in high efficiency of killing and may restrain the development of de novo colistin resistance. The dual potential mechanism of using the combination of colistin-rifampin to enhance bacterial killing activity implies the damage of bacterial OM by colistin, which facilitate rifampin penetration, as it does not effectively penetrate the OM in Gram-negative pathogens due to its hydrophobicity<sup>13</sup>. The combination treatment against a colistin-sensitive isolate completely inhibits the development of colistin-resistant populations in both vaccines<sup>34</sup>. Generally, the mode action of rifampin towards Gram-negative bacteria is to attenuate the penetration across the outer membrane rather than reducing sensitivity to the specific target<sup>35</sup>. In Gram-negative bacteria, where colistin primary targets the LPS of the outer membrane, electrostatic interaction between the amine-positive groups of colistin and the phosphate, anionic groups of the phosphate and carboxylate on the LPS is initiated. This reaction resulted in displacement of the original divalent cations and disruption of the membrane, and subsequently increased the permeability of the OM not only to itself (the so-called self-absorption) but also to other compounds<sup>36</sup>.

This may be attributed to intrinsic changes in the OM of *A. baumannii* linked with the emergence of resistance to colistin<sup>37,38</sup>, that facilitate the influx of these drugs to their final target sites within cells. Our results showed for the first time that the using of colistin and rifampin together, with such concentrations is clinically approachable, which significantly increased the killing spectrum of both colistin-resistant and colistin-resistant MDR and such that therapy prevented or inhibited the development of colistin-resistant bacterial community. Clinical studies are necessary to improve the combination treatment with colistin and rifampin.

Heterogeneous and complete resistance to colistin has been clinically reported<sup>39,40</sup>. The increasing use of colistin is believed to possess a risk of the emergence of new isolates that are resistant to this compound. In South Korea, levels of colistin resistance have been reported as high as

27.9 % , which indicted to the possibility of a potential emergence<sup>39</sup>.

These findings emphasize the need to identify the pathways that mediate colistin resistance in *A. baumannii*. Some colistin resistance mechanisms are associated with mutations that interrupt drug interaction with LPS. Colistin acts by rupturing the outer membrane, via necessarily attachment to the lipid part of the lipopolysaccharide (LPS). As a result, for example, resistance may occur via mutations in the PmrAB ;two-component system; which located downstream the target site, PmrC, catalyzes lipid A modification of phosphoethanolamine<sup>41</sup>.

This alteration decreases the colistin affinity for this subcellular target and the negative charge reduction of the outer membrane, so that mutations or insertions in the genes encoding the process of lipid biosynthesis mediate resistance by stopping the development of LPS and thereby removing the target of colistin<sup>38,40</sup>. In addition , the development of resistant isolates by adaptive mutations during antibiotic therapy in human patients is well known in *A. baumannii*<sup>42,43</sup>. Taken together, the results illustrate the ability of this organism to adapt and indicate that initial resistance towards AMPs therapy is mediated by genuine mechanisms in *A. baumannii*, which required adaptive mutations and resistance determinants. The molecular basis of the intrinsic resistance mechanisms in *A. baumannii* is largely unknown.

The use of combination treatment by two antibiotics reduces the concentration of each individual antibiotic that is used. On the other hand, the study showed that the majority of strains showed MIC value of 4 for rifampin (42 %) and MIC value of 2 for colistin (30%). Most notably that the MIC was measured independently for each antibiotic and then the sub-inhibitory concentration or lower MIC concentration was used to combine the two antibiotics. We therefore proposed a proportional experimental the combination of two antibiotics with a concentration of 2 µg ml<sup>-1</sup> for rifampin and 1 µg ml<sup>-1</sup> for colistin, based on our results, the concentration of AMPs is not arbiter and can be different, depends on the tolerance capacity of the XDR or MDR strains.

Previously, we showed that *A. baumannii* exhibits an increased resistance to colistin in response to physiological concentrations of monovalent cation<sup>44</sup>. Here we presented the discovery of more than 30 genes involved in colistin-induced tolerance in *A. baumannii*. The majority of these genes converge in mechanisms and systems involved in osmotic tolerance, including those elaborate in cell envelope

biosynthesis as well as in protein folding, such as *lpsB* gene.

#### Quantitative PCR Analysis (qPCR)

In this study, the expression level of *lptD*, *lptA* and *lptE* has been measured , and it varied within the two strains. The mRNA levels of *lptD*, *lptA* were decreased in both strains at 2 µg ml<sup>-1</sup> concentrations of colistin, but in HHR1 the levels were lower than in standard strain. In addition, this study demonstrates that expression and transcription of genes depends on the type and dose of antimicrobial agents and also on bacterial type (Fig. 6, 7)<sup>45</sup>. On the other hand, the mRNA levels of *lptE* were strongly increased in HHR1 in the presence of colistin compared with ATCC, and this strongly supports the detrimental role of colistin on *lptE* and in pathogenicity especially in clinical strain (Fig. 5). Moreover, other reports revealed that porins participate in increasing the virulence of *A. baumannii*, and others reported that the expression level of this gene is deferring among *A. baumannii* isolates.

The removal of *lptD* produces a complete loss of LPS and reduces polymyxin resistance in *A. baumannii*<sup>46</sup>, while the presence of *lpsB* protects *A. baumannii* from the bactericidal effect of colistin by encoding the glycosyltransferase that responsible for LPS synthesis<sup>47</sup>, Furthermore, high resistance to colistin phenotype in *A. baumannii* is attributed to single mutation in the *vacJ* (R166N)<sup>48</sup>.

The main factor in metabolism is biotin, and it has been reported as a critical factor corresponding to polymyxin sensitivity in *A. baumannii*, and the increase levels of biotin lead to increases the production of lipid A with a subsequent increase in colistin sensitivity. It was believed that knock out genes associated with biotin synthesis leads to a decrease in *A. baumannii*'s susceptibility to colistin<sup>47,49</sup>.

In the previous studies, a small molecule targeting MsbA was discovered; the regulation of LPS biosynthesis is not directed by MsbA inhibition in the organism, although the most frequent resistance mechanism that observed is the inhibition of LPS biosynthesis. It was believed that the disruption in the essential steps in a pathway, cells are unable to stop LPS biosynthesis. However, apart from *lpxACD*, disruption of non-essential steps regulates LPS biosynthesis.

In particular, inactivation of LptD leads to a significant decrease in LPS levels<sup>46</sup>, indicating the feedback response regulating LPS biosynthesis. In contrast, MsbA inhibition is unable to elicit the same response. It was speculated that the inhibition of MsbA increases the biosynthesis of LPS in the inner membrane, and changes the properties of the

membrane, and thus interferes with one or more of the basic processes that depend on their normal state. The dispersion of ATP or lacking the common precursors from other essential pathways, such as fatty acid biosynthesis, may exaggerate the inhibition of LPS.

The discovery of inhibitors of LPS biogenesis was challenging because the pathway included complex substrates made up of seven proteins and complex substrates with limited solubility. Using whole cells<sup>50</sup> indicates the feedback responses regulating LPS biosynthesis. In contrast, MsbA inhibition is unable to elicit the same response. It is speculated that the increase of LPS in the inner membrane resulting from MsbA inhibition changes the properties of the membrane, and thus interferes with one or more of the basic processes that depend on their normal state. This may be exacerbated by dissipation of ATP or depletion of common precursors from other essential pathways, such as fatty acid biosynthesis.

The discovery of inhibitors of LPS biogenesis was challenging because the pathway included complex substrates made up of seven proteins and complex substrates with limited solubility. Using whole cells, achieving a clearance of the LPS pathway confers resistance rather than sensitivity.

#### **Effect of Colistin on Adherence of *A. baumannii* on A-549 Cell**

The results showed that *A. baumannii* was able to invade and adhere to the A-549 cells because of the presence of OmpA protein which is responsible for the adhesion to epithelial cells, so when OmpA binds to cells, it causes mitochondrial dysfunction and promotes programmed cell death as shown in figure A-549 in which the cells were destroyed when they were invaded by *A. baumannii* strains (Fig. 8). Moreover, attraction depends on the bacterial strains, *A. baumannii* isolates harboring the blaPER-1 gene that increased the adhesion to epithelial cells, and then this gene plays an essential role in the association of *A. baumannii* with epithelial cells<sup>51</sup>.

Moreover, previous studies have described that the AbFhaB/ FhaC system in *A. baumannii* also has a role in binding to host cells because this structure binds to fibronectin which is the host receptor for *A. baumannii*<sup>52</sup>. Moreover, other studies defined that OmpA protein binds to the host proteins that mediate the cell in cell adhesions, so it is connected to the desmosome and causes its perturbation, which facilitate the adhesion to epithelial cells<sup>53</sup>. In this study, the cytotoxic effect of *A.baumannii*-colistin treated on human A549 alveolar cells has been described. The ability of *A. baumannii* isolated to infect the A549 respiratory alveolar cell was

evaluated using a microscope at 40X magnification. After incubation of the two strains of *A. baumannii* with A549 cells, the respiratory A549 cells were identified<sup>54</sup>.

Previous studies revealed that mixing *A.baumannii* ATCC 19606 WT strain with A549 cells reduces the viability of cells for about 65%. Similarly, when the A549 cells are incubated with *A. baumannii pmrB* mutant reduces the viability for about 57%, whereas, a slight decrease in A549 cell viability when they were incubated with both *lpx* mutants. Thus, *lpx* mutants exhibit an extremely low efficiency to complete inhibition of A549 respiratory cells<sup>54</sup>.

Furthermore, the effect of t LPS in pathogenic bacteria in response to radiotherapy in several NSCLC cell lines was investigated. The clinical background is given by noting that pneumococcal bacterial infections exacerbate the prognosis of patients with lung cancer. LPS is the main causative agent of Gram-negative bacteria, which appears predominantly in lung cancer patients<sup>55,56</sup>. The TLR-4 dependent pathways are activated by LPS, which also required the activity of EGFR protein kinase<sup>57</sup>, three NSCLC cell lines for non-small cell lung cancer were selected for very differential. In LPS response with A549 (EGFR wild type) TLR-4 expression and (EGFR deficiency) are shown.

Previous studies demonstrated that tumor growth in various experimental designs of NSCLC in vitro, ex vivo and in vivo was effectively induced by LPS, using the A549 cell line<sup>58</sup>. It is now observed that A549 colony formation ability is not affected by LPS. These results demonstrate a new aspect of LPS ability to modify the biology of NSCLC due to proliferation induction by LPS<sup>58</sup>, however, the reproduction of tumor cells is not affected by LPS. Cancer cell reproduction is an important factor in tumor progression, progression, and recurrence after treatment. The radiation sensitivity of cancer cells in vitro is known to correlate well with the tumor's response to in vivo radiotherapy, and thus, an association between the stem in vivo and in vitro cloning has been proposed indicating the importance of intrinsic radiation sensitivity to cancer. Stem cells for radiation sensitivity to tumors of different tissues<sup>59,60</sup>.

#### **The Half Inhibitory Concentration (IC<sub>50</sub>) of Colistin on *A. baumannii***

The IC<sub>50</sub> values of colistin can be calculated from the viability results to determine the inhibitory concentration of the *A. baumannii* and cytotoxicity concentration on *A. baumannii* (Fig. 9). The

concentration that inhibits 50% of cell growth was calculated by GraphPad Prism program.

### Conclusion:

The results conclude that the local isolate of *A. baumannii* HHR1 has been more virulence than ATCC17904. The effect of AMPs on *A. baumannii* increases by increasing the time of incubation, and the standard isolate is more sensitive to AMPs than HHR1. The effect of AMPs on *A. baumannii* has also been increased by increasing the concentration, and HHR1 is more resistant to AMPs than ATCC17904. Colistin affects the expression level of *lptD*, *lptE*, and *lptA* genes, also the results prove that the expression of *lptD*, *lptA* of *A. baumannii* ATCC 17904 is higher than the same genes in *A. baumannii* HHR1. While *lpt E* gene of the *A. baumannii* ATCC 17904 shows an up-regulation pattern than *A. baumannii* HHR1. Colistin affects the adhesion of *A. baumannii* on epithelial cells (lung cancer cell A-549).

### Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

### Authorship contribution statement:

Title of manuscript: Colistin as A Good Monotherapy to Restrain the Pathogenicity of *Acinetobacter baumannii* In vivo and In vitro (NS was responsible for collecting the data from each assay or experiment, and analyzing the data as a part of her MSc project) (HH contributed in conception, design, acquisition of data, analysis, interpretation, drafting the MS, revision and proofreading)

### References:

1. Al-Haideri HH. Gene Expression of blaOXA-51-like and blaOXA-23 in response to  $\beta$ -Lactam antibiotic in clinically isolates *Acinetobacter baumannii* and *Acinetobacter baumannii* from urine samples. Iraqi J Agric Sci. 2019;50(4).
2. Rebic V, Masic N, Teskeredzic S, Aljicevic M, Abduzaimovic A, Rebic D. The importance of acinetobacter species in the hospital environment. Med Arch. 2018;72(5):325.
3. Van Duin D, Paterson DL. Multidrug-resistant bacteria in the community: trends and lessons learned. Infectious Disease Clinics. 2016 Jun 1;30(2):377-90.
4. Xu L, Shao C, Li G, Shan A, Chou S, Wang J, Ma Q, Dong N. Conversion of broad-spectrum antimicrobial peptides into species-specific antimicrobials capable of precisely targeting pathogenic bacteria. Sci. Rep. 2020 Jan 22;10(1):1-9. *اختصار اسم المجلة*
5. Zanetti M. Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol. 2004;75(1):39-48.
6. Lei J, Sun L, Huang S, Zhu C, Li P, He J, et al. The antimicrobial peptides and their potential clinical applications. Am J Transl Res. 2019;11(7):3919.
7. Dong H, Zhang Z, Tang X, Paterson NG, Dong C. Structural and functional insights into the lipopolysaccharide ABC transporter LptB 2 FG. Nat Commun. 2017;8(1):1-11.
8. Janssen AB, Bartholomew TL, Marciszewska NP, Bonten MJ, Willems RJ, Bengoechea JA, et al. Nonclonal Emergence of Colistin Resistance Associated with Mutations in the BasRS Two-Component System in Escherichia coli Bloodstream Isolates. Msphere. 2020 Apr 29;5(2).
9. Bialvaei AZ, Samadi Kafil H. Colistin, mechanisms and prevalence of resistance. Curr Med Res Opin. 2015;31(4):707-21.
10. Biswas S, Brunel J-M, Dubus J-C, Reynaud-Gaubert M, Rolain J-M. Colistin: an update on the antibiotic of the 21st century. Expert Rev Anti Infect Ther. 2012;10(8):917-34.
11. Li Z, Cao Y, Yi L, Liu JH, Yang Q. Emergent polymyxin resistance: end of an era?. In Open forum infectious diseases 2019 Oct (Vol. 6, No. 10, p. ofz368). US: Oxford University Press.
12. Bialvaei AZ, Kafil HS, Asgharzadeh M, Yousef Memar M, Yousefi M. Current methods for the identification of carbapenemases. J Chemother. 2016;28(1):1-19.
13. Lee HJ, Bergen PJ, Bulitta JB, Tsuji B, Forrest A, Nation RL, et al. Synergistic activity of colistin and rifampin combination against multidrug-resistant *Acinetobacter baumannii* in an in vitro pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother. 2013;57(8):3738-45.
14. Leelasupasri S, Santimaleeworagun W, Jitwasinkul T. Antimicrobial susceptibility among colistin, sulbactam, and fosfomycin and a synergism study of colistin in combination with sulbactam or fosfomycin against clinical isolates of carbapenem-resistant *Acinetobacter baumannii*. J Pathog. 2018;2018.
15. Hamzah AS. Genetic Variations in rplB Gene Associated with Multidrug Resistance *Acinetobacter baumannii* Isolated from Different Clinical Sources. Al-Nahrain J Sci. 2018;21(3):119-25.
16. Al-Haideri H. Characterization of novel proteins in the cell envelope of *Campylobacter jejuni* A thesis submitted in part fulfilment for the degree of Doctor of Philosophy. Department of Molecular Biology and Biotechnology, The University of Sheffield. 2015;(March).
17. Kadhim WKA, Nayef UM, Jabir MS. Polyethylene

- glycol-functionalized magnetic (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles: A good method for a successful antibacterial therapeutic agent via damage DNA molecule. *Surf Rev Lett*. 2019;26(10):1–15.
18. Al-Shammari AM, Alshami MA, Umran MA, Almukhtar AA, Yaseen NY, Raad K, et al. Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient. *Breast Cancer Targets Ther*. 2015;7:223.
  19. Aghapour Z, Gholizadeh P, Ganbarov K, Bialvaei AZ, Mahmood SS, Tanomand A, et al. Molecular mechanisms related to colistin resistance in Enterobacteriaceae. *Infect Drug Resist*. 2019;12:965.
  20. Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2006;50(9):2946–50.
  21. Hernan RC, Karina B, Gabriela G, Marcela N, Carlos V, Angela F. Selection of colistin-resistant *Acinetobacter baumannii* isolates in postneurosurgical meningitis in an intensive care unit with high presence of heteroresistance to colistin. *Diagn Microbiol Infect Dis*. 2009;65(2):188–91.
  22. Tan C-H, Li J, Nation RL. Activity of colistin against heteroresistant *Acinetobacter baumannii* and emergence of resistance in an in vitro pharmacokinetic/pharmacodynamic model. *Antimicrob Agents Chemother*. 2007;51(9):3413–5.
  23. Yu X-H, Song X-J, Cai Y, Liang B-B, Lin D-F, Wang R. In vitro activity of two old antibiotics against clinical isolates of methicillin-resistant *Staphylococcus aureus*. *J Antibiot (Tokyo)*. 2010;63(11):657–9.
  24. Bai Y, Liu B, Wang T, Cai Y, Liang B, Wang R, et al. In vitro activities of combinations of rifampin with other antimicrobials against multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2015 Mar 1;59(3):1466–71.
  25. Vázquez-López R, Solano-Gálvez SG, Juárez Vignon-Whaley JJ, Abello Vaamonde JA, Padró Alonzo LA, Rivera Reséndiz A, et al. *Acinetobacter baumannii* Resistance: A Real Challenge for Clinicians. *Antibiotics*. 2020 Apr;9(4):205.
  26. Asif M, Alvi IA, Rehman SU. Insight into *Acinetobacter baumannii*: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. *Infect. and drug resist*. 2018;11:1249.
  27. Krishnamurthy M, Lemmon MM, Falcinelli EM, Sandy RA, Dootz JN, Mott TM, et al. Enhancing the antibacterial activity of polymyxins using a nonantibiotic drug. *Infec. and Drug Resist.* 2019;12:1393.
  28. Kheshti R, Pourabbas B, Mosayebi M, Vazin A. In vitro activity of colistin in combination with various antimicrobials against *Acinetobacter baumannii* species, a report from South Iran. *Infec. and Drug Resist.* 2019;12:129.
  29. Giannouli M, Di Popolo A, Durante-Mangoni E, Bernardo M, Cuccurullo S, Amato G, et al. Molecular epidemiology and mechanisms of rifampicin resistance in *Acinetobacter baumannii* isolates from Italy. *Int J Antimicrob Agents*. 2012;39(1):58–63.
  30. Cheah S-E, Li J, Tsuji BT, Forrest A, Bulitta JB, Nation RL. Colistin and polymyxin B dosage regimens against *Acinetobacter baumannii*: differences in activity and the emergence of resistance. *Antimicrob Agents Chemother*. 2016;60(7):3921–33.
  31. Rigatto MH, Oliveira MS, Perdigão-Neto L V, Levin AS, Carrilho CM, Tanita MT, et al. Multicenter prospective cohort study of renal failure in patients treated with colistin versus polymyxin B. *Antimicrob Agents Chemother*. 2016;60(4):2443–9.
  32. Parker SL, Frantzeskaki F, Wallis SC, Diakaki C, Giamarellou H, Koulenti D, et al. Population pharmacokinetics of fosfomycin in critically ill patients. *Antimicrob Agents Chemother*. 2015;59(10):6471–6.
  33. Nastro M, Rodríguez CH, Monge R, Zintgraff J, Neira L, Rebollo M, et al. Activity of the colistin–rifampicin combination against colistin-resistant, carbapenemase-producing Gram-negative bacteria. *J Chemother*. 2014;26(4):211–6.
  34. Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microbiol Rev*. 2017 Apr 1;30(2):557–96.
  35. Wehrli W. Rifampin: mechanisms of action and resistance. *Rev Infect Dis*. 1983;5(Supplement\_3):S407–11.
  36. Hancock REW, Chapple DS. Peptide antibiotics. *Antimicrob Agents Chemother*. 1999;43(6):1317–23.
  37. Cafiso V, Stracquadanio S, Lo Verde F, Gabriele G, Mezzatesta ML, Caio C, et al. Colistin resistant *Acinetobacter baumannii*: genomic and transcriptomic traits acquired under colistin therapy. *Front Microbiol*. 2019 Jan 7;9:3195.
  38. Henry R, Vithanage N, Harrison P, Seemann T, Coutts S, Moffatt JH, et al. Colistin-resistant, lipopolysaccharide-deficient *Acinetobacter baumannii* responds to lipopolysaccharide loss through increased expression of genes involved in the synthesis and transport of lipoproteins, phospholipids, and poly-β-1, 6-N-acetylglucosamine. *Antimicrob Agents Chemother*. 2012;56(1):59–69.
  39. Ko KS, Suh JY, Kwon KT, Jung S-I, Park K-H, Kang CI, et al. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. *J Antimicrob Chemother*. 2007;60(5):1163–7.
  40. Park YJ, Hong DJ, Yoon EJ, Kim D, Choi MH, Hong JS, et al. Differences in Colistin-resistant *Acinetobacter baumannii* Clinical Isolates Between Patients With and Without Prior Colistin Treatment. *Annals of laboratory medicine*. 2018 Nov;38(6):545–54.
  41. Adams MD, Nickel GC, Bajaksouzian S, Lavender H, Murthy AR, Jacobs MR, et al. Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the PmrAB two-component system. *Antimicrob Agents Chemother*. 2009;53(9):3628–34.

42. López-Rojas R, Jiménez-Mejías ME, Lepe JA, Pachón J. Acinetobacter baumannii resistant to colistin alters its antibiotic resistance profile: a case report from Spain. *J Infect Dis.* 2011;204(7):1147–8.
43. Vrancianu CO, Pelcaru CF, Alistar A, Gheorghe I, Marutescu L, Popa M, et al. Escaping from ESKAPE. Clinical Significance and Antibiotic Resistance Mechanisms in Acinetobacter baumannii: a Review. *Biointerface Research in Applied Chemistry. Biointerface Reserch in Applied chemistry.* 2020 jul 11(1),8190-8203.
44. Hood MI, Jacobs AC, Sayood K, Dunman PM, Skaar EP. Acinetobacter baumannii increases tolerance to antibiotics in response to monovalent cations. *Antimicrob Agents Chemother.* 2010;54(3):1029–41.
45. He X, Lu F, Yuan F, Jiang D, Zhao P, Zhu J, et al. Biofilm formation caused by clinical Acinetobacter baumannii isolates is associated with overexpression of the AdeFGH efflux pump. *Antimicrob Agents Chemother.* 2015;59(8):4817–25.
46. Bojkovic J, Richie DL, Six DA, Rath CM, Sawyer WS, Hu Q, et al. Characterization of an Acinetobacter baumannii lptD deletion strain: permeability defects and response to inhibition of lipopolysaccharide and fatty acid biosynthesis. *J Bacteriol.* 2016;198(4):731–41.
47. Whitfield C, Trent MS. Biosynthesis and export of bacterial lipopolysaccharides. *Annu Rev Biochem.* 2014;83:99–128.
48. Nhu NTK, Riordan DW, Nhu TDH, Thanh DP, Thwaites G, Lan NPH, et al. The induction and identification of novel Colistin resistance mutations in Acinetobacter baumannii and their implications. *Sci Rep.* 2016;6(1):1–8.
49. El-Sayed Ahmed MAE-G, Zhong L-L, Shen C, Yang Y, Doi Y, Tian G-B. Colistin and its role in the Era of antibiotic resistance: an extended review (2000–2019). *Emerg Microbes Infect.* 2020;9(1):868–85.
50. Zhang G, Baidin V, Pahil KS, Moison E, Tomasek D, Ramadoss NS, et al. Cell-based screen for discovering lipopolysaccharide biogenesis inhibitors. *Proc Natl Acad Sci.* 2018;115(26):6834–9.
51. Longo F, Vuotto C, Donelli G. Biofilm formation in Acinetobacter baumannii. *New Microbiol.* 2014;37(2):119–27.
52. Pérez A, Merino M, Rumbo-Feal S, Álvarez-Fraga L, Vallejo JA, Beceiro A, et al. The FhaB/FhaC two-partner secretion system is involved in adhesion of Acinetobacter baumannii AbH12O-A2 strain. *Virulence.* 2017;8(6):959–74.
53. Schweppe DK, Harding C, Chavez JD, Wu X, Ramage E, Singh PK, et al. Host-microbe protein interactions during bacterial infection. *Chem Biol.* 2015;22(11):1521–30.
54. Nie D, Hu Y, Chen Z, Li M, Hou Z, Luo X, et al. Outer membrane protein A (OmpA) as a potential therapeutic target for Acinetobacter baumannii infection. *Int J Biomed Sc.* 2020 Dec 1;27(1):26.
55. Berghmans T, Sculier J-P, Klastersky J. A prospective study of infections in lung cancer patients admitted to the hospital. *Chest.* 2003;124(1):114–20.
56. Pietrocola G, Arciola CR, Rindi S, Di Poto A, Missineo A, Montanaro L, et al. Toll-like receptors (TLRs) in innate immune defense against Staphylococcus aureus. *Int J Artif Organs.* 2011;34(9):799–810.
57. Chattopadhyay S, Veleparambil M, Poddar D, Abdulkhalek S, Bandyopadhyay SK, Fensterl V, et al. EGFR kinase activity is required for TLR4 signaling and the septic shock response. *EMBO Rep.* 2015;16(11):1535–47.
58. Hattar K, Savai R, Subtil FSB, Wilhelm J, Schmall A, Lang DS, et al. Endotoxin induces proliferation of NSCLC in vitro and in vivo: role of COX-2 and EGFR activation. *Cancer Immunol Immunother.* 2013;62(2):309–20.
59. Malaise EP, Fertil B, Chavaudra N, Guichard M. Distribution of radiation sensitivities for human tumor cells of specific histological types: comparison of in vitro to in vivo data. *Int J Radiat Oncol Biol Phys.* 1986;12(4):617–24.
60. Krause M, Dubrovska A, Linge A, Baumann M. Radioresistance, prediction of radiotherapy outcome and specific targets for combined treatments. *Adv Drug Deliv Rev.* 2017;109:63–73.

## عقار الكولستين علاج احادي جيد لكبح جماح بكتريا الراكدة البومانية *Acinetobacter baumannii* خارج وداخل الجسم الحي

هاله هيثم الحيدري

نغم سعدي محمد

قسم علوم الحياة، كلية العلوم للنبات، جامعة بغداد، بغداد، العراق

### الخلاصة :

تعد بكتيريا *Acinetobacter baumannii* من احد مسببات الأمراض الانتهازية الرئيسية التي تقاوم العديد من المضادات الحيوية. الكولستين هو مضاد حيوي يستخدم في الوقت الحاضر كطريقة لمعالجة أخيرة للعزلات ذات المقاومة الشديدة. الغرض من هذه الدراسة هو التعرف على أهمية جين LptD في الطبقة الدهنية LPS من بكتريا *A. baumannii* ومعرفة دورها الاساسي كعامل ضراوة. في الدراسة الحالية، تم استخدام عزلتين من بكتريا *A. baumannii*، العزلة المحلية HHR1 والعزلة العالمية ATCC 17904، وتم استخدام ثلاث انواع من المضادات الحيوية (الكولستين، الريفامبيسين و الفوسفومييسين كعلاج احادي او مزدوج خارج وداخل الجسم الحي. اظهرت النتائج أن HHR1 *A. baumannii* هي أكثر مقاومة لـ AMPs (Antimicrobial peptides) من السلالات العالمية، تم ملاحظة ازدياد تأثير AMPs على *A. baumannii* بزيادة التركيز ووقت الحضانة، كما تبين أيضاً أن AMPs تعتبر قاتله على بكتريا *A. baumannii* عند التركيز العالي ( $2 \mu\text{g ml}^{-1}$ ) في العلاج الأحادي و ( $1.5, 2 \mu\text{g ml}^{-1}$ ) في الدمج. علاوة على ذلك، كانت حساسية العزلات متغايرة، حيث اظهر الكولستين فعالية متميزة في تثبيط النمو البكتيري كعلاج احادي او عند المزج مع مضاد اخر. اظهرت النتائج أن التعبير الجيني لجينات *lptA*، *lptD* للعزلة المحلية أقل من العزلة القياسية بتركيز  $2 \mu\text{g ml}^{-1}$  من الكولستين، بينما جين *lptE* كانت العزلة القياسية ذات نمط تنظيم أعلى مما كان عليه في العزلة المحلية. علاوة على ذلك، يؤثر الكولستين على التصاق *A. baumannii* على الخلايا الظهارية (A-549 خلية سرطان الرئة)، وأظهر الكولستين تأثيراً كبيراً على نمو البكتيريا، وتكاثر الخلايا، عن طريق تقليل اعداد البكتيريا.

الكلمات المفتاحية : المضادات البكتيرية البيبتيدية، العديد السكري الدهني، تفاعل البلمرة المتسلسل، *Acinetobacter baumannii*.