INTERNATIONAL JOURNAL OF HEALTH & MEDICAL RESEARCH

ISSN(print): 2833-213X, ISSN(online): 2833-2148

Volume 04 Issue 12 December 2025

DOI : 10.58806/ijhmr.2025.v4i12n07

Page No. 683 - 688

Influence of Kaempferol on Biofilm Formation and Adhesion-Related Gene Activity in MRSA

Fouad Qasim Jubair Al-Zayadi[1*], Mohammed Mutashar Yasir[2]

[1,2]Department of Biology, College of Education for Pure Sciences, Al-Muthanna University, Samawah, Iraq

ABSTRACT:

Background: The widespread methicillin-resistant *Staphylococcus aureus* (MRSA) dissemination poses significant public health challenges, particularly because of limited elimination options for related infections. the present study antimicrobial effects of the natural compound Kaempferol against MRSA, singly and in combination with others (such as metformin), and examined its impact on the expression of key virulence genes associated to MRSA pathogenicity.

Methods: Ten MRSA isolates were collected from clinical specimens of 12–55 years-aged patients. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs), of the agents were tested within a range of 0.5–1024 µg/mL. Anti-biofilm effects were assessed using a microplate tissue assay. The expression levels of selected virulence genes (*spa*, *fnbA* and *pvl*) were quantified by real-time PCR following exposure to the agents.

Results: There was a synergistic and additive antibacterial effects between kaempferol and metformin. Also, metformin and kaempferol had anti-biofilm effects. Kaempferol at sub-MIC concentration could decrease the expression of *fnbA*, *pvl*, and *spa* genes by mean ±SD of 1.6 ±0.6 ($p<0.05$), 1.8 ±0.1 ($p<0.05$), and 1.2 ±0.4 fold ($p<0.05$), respectively in comparison with control. The effect of metformin at sub-MIC level was also significant on these genes including 1.9±0.6 ($p<0.05$), 2.2 ±0.3 ($p<0.05$), and 1.9 ±0.4 fold ($p<0.05$), respectively.

Conclusion: Kaempferol and metformin had antibacterial and anti-biofilm activities against MRSA isolates. Further studies with larger numbers of isolates and detailed molecular mechanistic research is warranted to confirm findings.

KEYWORDS: Staphylococcus aureus Methicillin resistance, Virulence genes, Kaempferol, Antibiotic synergy

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) presents several challenges for public health and healthcare systems around the world, whether acquired in clinical or community settings [1]. MRSA is capable of developing resistance to many classes of antibiotics and poses a growing risk to human health. Among the characteristics attributed to the virulence of MRSA are its ability to form biofilms and express various adhesins that assist in colonisation, immune evasion and the persistence of infections [2]. The presence of biofilm-forming microorganisms provides additional protection against both antimicrobial therapy and the host immune defence mechanisms that contribute to the occurrence of chronic and device-associated (e.g., endocarditis, osteomyelitis and chronic wound) infections. Understanding the molecular mechanisms involved in biofilm formation and adhesion of S. aureus, especially those that involve MRSA strains, are critical to developing new therapeutic approaches for treating infections caused by MRSA.

The process of biofilm formation by MRSA isolates involves four distinct stages: 1) initial contact and attachment of the bacteria to the host or abiotic surface; 2) accumulation of bacterial cells to form a layer; 3) the thickening of the biofilm layer; and 4) the dispersal of bacterial cells from the biofilm layer to form additional biofilm layers on other surfaces. The bacterial adherence to host or abiotic surfaces is mediated by a class of proteins known as surface adhesins (MSCRAMMs) [2, 3]. The MSCRAMM genes pvl, fnbA and spa code for proteins that permit attachment to components of the extracellular matrix of the host, as well as to abiotic surfaces, thereby facilitating the onset and the progress of biofilm growth. Adhesins are controlled globally by several factors (global regulators), including the SarA, Agr, and two-component regulatory systems that are activated by environmental signals and alter the expression of specific genes during various stages of the infectious process. The emergence of antibiotic-resistant bacteria necessitates the search for alternative antimicrobial agents and methods to prevent and treat biofilms [4]. Compounds found in nature, particularly polyphenolic compounds (such as flavonoids), have attracted much attention due to their potential as universal antimicrobials, their very low toxicity, and the ability to disrupt virulence factors in pathogenic bacteria. The flavonoid kaempferol (a natural flavonol) is found in a variety of edible fruits, vegetables, and medicinal herbs and has been shown to exhibit antibacterial action against a variety of bacterial pathogens, with antimicrobial activity mediated by the following mechanisms: disruption of the

integrity of the bacterial cell membrane, inhibition of enzyme activity, and disruption of the process of quorum sensing. Recent studies have shown that kaempferol exhibits anti-biofilm activity against a number of different bacterial species (i.e., *Pseudomonas aeruginosa* and Escherichia coli) [5, 6]. However, much more research needs to be done to determine its effects on the biofilm-forming ability of *Staphylococcus aureus*, especially methicillin-resistant strains (MRSA), and on the expression of specific virulence-related genes. Flavonoids have the potential to affect biofilm formation through multiple mechanisms; for example, they can inhibit or impair early adhesion and/or disrupt established biofilms and/or regulate the expression of virulence associated genes. These activities are particularly important in the context of MRSA infections, where biofilm Iamong other factors pose great challenges for treatment and require novel approaches.

The information gained from this study regarding how kaempferol will impact MRSA biofilm formation, as well as the impact of kaempferol on the expression of MRSA adhesin genes, could provide insights that lead to the development of adjunct therapies to complement existing antimicrobial therapies or serve as stand-alone anti-virulence therapeutics. Natural compounds such as kaempferol may also provide novel ways to reduce bacterial colonization and survival, which can reduce the severity and spread of infection by acting on multiple biofilm- and adhesion-related pathways [7-9]. Finally, understanding the molecular-level interaction between kaempferol and bacterial virulence determinants could identify new therapeutic targets for antimicrobial intervention and develop new therapies for the treatment of biofilm-associated infections.

The purpose of the present study is to evaluate the effect of kaempferol on MRSA biofilm formation and the expression of key adhesin genes from MRSA isolates. This research will contribute to our understanding of how flavonoids may act as anti-virulence agents and support the development of alternative and supplemental strategies for the treatment of antibiotic-resistant bacterial infections. The ongoing problem of MRSA infection is forcing researchers to develop novel approaches to prevent the formation of biofilms and to inhibit the adhesion of MRSA. Natural products such as kaempferol provide new options for this aim because they possess a variety of biological functions. The purpose of this study is to conduct an in-depth investigation to determine how kaempferol affects the formation of biofilms and the expression of the adhesin genes in MRSA, thereby creating a foundation to develop novel therapies to combat biofilm formation and improve clinical scenarios.

MATERIALS AND METHODS

Bacterial Isolates and Sources

The study analysed a total of 10 MRSA samples obtained from participants with an age range of 12 to 55 years hospitals of Al-Muthanna province. For testing their susceptibility toward certain antimicrobials; the samples were cultured in Mueller-Hinton Broth (MHB) and Luria-Bertani (LB) media.

Preparation of Kaempferol

Kaempferol (≥99% purity) (Sigma Aldrich) was dissolved in dimethyl sulfoxide (DMSO) to develop stock solutions of Kaempferol. Subsequent to diluting the stock in MHB to create working solutions, the total DMSO concentration was kept lower than 1% (v/v) (20). To confirm that bacterial death had occurred, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Kaempferol were determined by taking aliquots from wells with no observable growth and stamping them onto LB agar for verification of death.

Antibacterial Susceptibility Testing

To evaluate the antibiotic susceptibility of the MRSA strains, both the disc diffusion and broth microdilution techniques were employed. For all isolates, results confirmed resistance to beta-lactam antibiotics, particularly methicillin and oxacillin. MIC and MBC values were determined following the guidance of CLSI (Clinical and Laboratory Standards Institute), with a range from 0.125 to 1024 µg/mL. Standard strains of *S. aureus* ATCC 25923 and MRSA reference strains were used as controls for evaluation of synergistic antibacterial activity of Kaempferol and Metformin in combinations against MRSA.

The synergistic antibacterial activity of Kaempferol in combination with Metformin against MRSA was evaluated using the checkerboard microdilution assay. A sterile 96-well microtiter plate was prepared with a two-dimensional matrix; one axis was a series of dilutions of Imipenem and the other axis was a series of dilutions of the individual agent (Kaempferol or Metformin).

Kaempferol was tested from 1 to 1024 µg/mL and Imipenem was tested from sub-inhibitory to inhibitory concentrations. A standardized MRSA strain was inoculated onto each plate at approximately 5×10^5 CFU/mL and incubated for 24 hours at 37°C. The MIC for each treatment alone and in combination was determined visually and using a spectrophotometer at 600 nm.

Interactions of Imipenem with each of the test agents was quantified by determining the Fractional Inhibitory Concentration Index (FICI) for the agent in combination with Imipenem. The FICI was determined using the following criteria: FICI ≤ 0.5 indicates synergy; 0.5 < FICI ≤ 1 indicates an additive effect; 1 < FICI ≤ 4 indicates indifference; FICI > 4 indicates antagonism. Control wells included wells containing either antibiotic alone, compound alone, water, or positive control strains of MRSA. MRSA Virulence Gene Expression - Total RNA was isolated from MRSA strains incubated for four hours with sub-MIC (0.5 MIC) concentrations of Kaempferol and Metformin by using the TRIzol RNA Isolation Reagent (from InvivoGen) following the manufacturer's instructions. All RNA samples were treated for decontamination, using DNase I.

Forward and reverse specific primer pairs were designed based on selected MRSA virulence genes, including *fnbA* (fibronectin-binding protein A), *pvl* (Panton-Valentine leukocidin), and *spa* (protein A) as following:
fnbA: F: TGCAGGAACAGGTTGCTTGT, R: GCTGTTGAGGTTGGTGTTGG
pvl: F: ATCATTGAACGCTGACTTGC, R: GCTTGAACGAGATATTATCG
spa: F: GAGATGAACGCTGTTGTTGG, R: CAGGTTCTTGCTTTGTTGGC
For real-time quantitative PCR, the primers were employed in individual reactions with the following components: 12.5 µL of SYBR® green real-time PCR master mix (Applied Biosystems), 3 µL of cDNA template from the MRSA treatment, and 1 µL of each specific primer; the total volume per reaction was adjusted to 25 µL with the addition of RNase-free water. The reactions were amplified using a Rotor-Gene Q 5 Plex System (Qiagen) with thermal cycling conditions consisting of an initial denaturation step at 95°C for three minutes, followed by 40 cycles at 95°C for 15 seconds of denaturation, 60°C for 30 seconds of annealing, and 72°C for 30 seconds of extension.
Gene expression levels were quantified relative to the housekeeping gene *gyrB* using the $2^{-\Delta\Delta Ct}$ method. The results were expressed as fold change in gene expression compared to untreated control strains.
The microtiter plate assay was used to evaluate biofilm production. A 96-well plate was developed with nutrient broth media containing bacterial cultures. The bacterial suspension was diluted to an OD_{600} of 0.1 and 100 µL of the diluted suspension was put into each well of the plate. After this addition, the plate was incubated for 24 hours. The control wells contained the media without any bacteria.
After the incubation period, the media was removed from the wells and two separate washes of sterile saline were performed on each well to get rid of any non-adherent cells. To visualize the biofilms, 100 µL of a 0.1% solution of crystal violet was added to each well and then incubated at room temperature for approximately 15 minutes. The wells were subsequently washed with sterile saline and left to air dry for approximately 24 hours after washing. To solubilize the dye bound to the biofilms, 96% ethanol was added to each well and the absorbance of the resulting solution was read at 570 nm using a microplate reader.
To assess anti-biofilm activity, the level of biofilm development in the untreated/control wells was compared to that of the metformin- and kaempferol-treated wells. The experiments were conducted in groups of three. All biofilm-producing bacteria were classified by their optical density (OD): OD ≤ 0.08 indicates a lack of production of biofilms, whereas an OD from 0.08 to 0.32 represents a high-level biofilm producer. A cut off point for OD (ODc) was found as follows: ODc= 0.05 + (3 x 0.01) = 0.08; 0.01 was in reference to the standard deviation.
Using this classification system, the categories of biofilm producers were established:
- Non-biofilm producer: OD ≤ ODc (≤ 0.08)
- Weak biofilm producer: ODc < OD ≤ 2 × ODc (0.08–0.16)
- Moderate biofilm producer: 2 × ODc < OD ≤ 4 × ODc (0.16–0.32)
- Strong biofilm producer: OD > 4 × ODc (> 0.32)

Data analysis was completed using one-way ANOVA with Tukey's post hoc test and Chi square tests done on GraphPad Prism 8.1 with p-value< 0.05 considered statistically significant.

RESULTS

Antibiotic Susceptibility

MRSA were isolates from respiratory (n=6) and skin (n=4) infections. MRSA isolates were resistant to tetracycline, cefoxitin, ciprofloxacin and erythromycin. Also, resistance rate was considerably high for gentamicin and piperacillin-tazobactam, each being 60%, and for amikacin at 50%. Moreover, resistance rate for vancomycin, linezolid, and nitrofurantoin were 30%, 10%, and 5%, respectively.

MIC/MBC Levels

MRSA isolates showed cefoxitin MIC and MBC ranges of 32–64 µg/mL and 32–128 µg/mL, respectively. In addition, these ranges for kaempferol were respectively 128-512 µg/mL and 512->1024 µg/mL. Moreover, the MIC and MBC ranges of metformin included and 128-256 and 256-512, respectively. There was synergistic and additive antibacterial effects in the combination of Kaempferol and metformin which decreased MIC and MBC ranges of metformin. The findings of interactions between Kaempferol and metformin has been shown in Table 1.

Table 1. Synergistic effects of Kaempferol and metformin

Isolates	Kaempferol MIC (µg/mL)	Metformin MIC (µg/mL)	Kaempferol FIC	Metformin FIC	Imipenem FICI
1	128	128	64/128=0.5	64/128=0.5	1
2	128	256	64/128=0.5	64/256=0.25	0.75
3	512	256	128/512=0.25	128/256=0.5	0.75

4	256	128	128/256=0.5	32/128=0.25	0.75
5	256	128	128/256=0.5	32/128=0.25	0.75
6	128	256	64/128=0.5	128/256=0.5	1
7	256	256	128/256=0.5	64/256=0.25	0.75
8	128	512	64/128=0.5	256/512=0.5	1
9	512	512	128/512=0.25	128/512=0.25	0.5
10	256	512	128/256=0.5	256/512=0.5	1

Biofilm Formation

The moderate level of biofilm formation was observed among MRSA isolates showing OD values between 0.169±0.066 and 0.3203±0.041, in comparison to mean control wells OD with 0.093. There was a decrease in the levels of biofilm formation in combination of sub-MIC concentrations of Kaempferol and metformin at 64 µg/mL to weak and no biofilms levels demonstrating the ability of metformin and Kaempferol biofilm inhibitory properties.

Gene Expression

Among MRSA isolates, Kaempferol at sub-MIC concentration could decrease the expression of *fnbA*, *pvl*, and *spa* genes by mean ±SD of 1.6 ±0.6 ($p<0.05$), 1.8 ±0.1 ($p<0.05$), and 1.2 ±0.4 fold ($p<0.05$), respectively compared to the control. The effect of metformin at sub-MIC level was also significant on these genes including 1.9±0.6 ($p<0.05$), 2.2 ±0.3 ($p<0.05$), and 1.9 ±0.4 fold ($p<0.05$), respectively.

DISCUSSION

Kaempferol's inhibitory effects against MRSA includes antibacterial activity and its effect on the virulence factor(s), as well as a reduction in biofilm production by MRSA following exposure to Kaempferol and metformin [5, 6, 9]. Previous studies showed that Kaempferol and metformin possess anti-biofilm activity, including Kaempferol's ability to inhibit the formation and disrupt the quorum sensing of the biofilm of certain bacterial pathogens [10, 11] and *Candida albicans* [12, 13]. Thus, the evidence suggests that Kaempferol's anti-biofilm activity applies to multiple strains of bacteria, including MRSA. Additionally, we found that Kaempferol and metformin decreased the expression of three adhesin genes (*pvl*, *spa*, and *fnbA*), affecting their expression. Because these genes are responsible for encoding the adhesin(s) necessary for MRSA virulence, this finding is critical toward uncovering MRSA virulence.

The mechanisms through which Kaempferol and metformin function are different and thus give different results. The effectiveness of Kaempferol in fighting infection comes from its ability to damage the membrane of bacteria instead of simply interfering with the formation of the cell wall, as is the case for imipenem. Whereas, Kaempferol may affect the whole physiological function of a bacterium by damaging the membrane and disrupting the processes of gene expression, metformin probably acts in a more limited manner. Because of the numerous complexities of how bacteria produce and regulate their gene expression, and regulatory mechanisms, such as two-component regulation, pathways of signal transduction, and networks of the global regulatory, that involve the integrity of the membrane, enzymes, modification of nucleic acids, the specific nature of how either drug affects virulence-associated genes remains to be fully identified [14-16]. The death of bacteria caused by either drug may also provide a further reduction of the bacterial population, leading to a reduction of virulence gene expression due to lower levels of adhesin expression. Metformin demonstrated antibacterial effects on MRSA, based on MIC and MBC, but could prevent biofilm formation by MRSA. More studies are needed to investigate how metformin regulates biofilm formation at the molecular level. Furthermore, metformin exhibits anti-biofilm activity against a variety of fungal and bacterial pathogens [17, 18].

Membrane damage/disruption will influence lots of other things e.g., through (but not limited to) altering cellular stress-response pathways (e.g., CpxAR, RpoE, BaeSR), the repair pathways (e.g., envZ/ompR), and global regulatory networks (e.g., AcrAB-TolC), as well as changing the structure or composition of proteins, and the charge of the cell surface; all of these changes could lead to the deregulation of the expression of numerous genes [19-22]. Several studies have exhibited the antibacterial and anti-biofilm effects of kaempferol in combination and formulated in nanoparticles [7-9]. Metaformin has considerable impact on the inhibition of biofilms of MRSA isolates by converting biofilm formation from moderate to weak, or no biofilm formation. At sub-inhibitory levels of metaformin (1/10 of MIC = 10 mg/ml), a 67.9% decrease in biofilm growth by *P. aeruginosa* PAO1 was achieved [23]. Also, two evidences demonstrated biofilm inhibition of *P. aeruginosa* by 67% and 43.8% when treated at sub-MIC levels [24, 25]. Moreover, the amount of 22%-68% reduction in biofilm formation was also demonstrated with *K. pneumoniae* [26].

These studies and others herein support Kaempferol as a candidate for future investigations to use in conjunction with approved drugs such as antibiotics, as a potential alternative to treat infections caused by antibiotic resistant MRSA, as Kaempferol can inhibit biofilm formation as well as reduce the expression of virulence related genes - and thus provide an additional way to manage infections caused by antibiotic resistant organisms [36]. Kaempferol offers a new approach to address skin and respiratory cases caused by multidrug-resistant organisms (MDRO) because of important properties of blocking biofilm development and down-

regulating virulence-associated gene expression. Combination therapies, including kaempferol, may be developed to lessen the total dosage of antibiotics utilized or to delay the onset of drug resistance.

CONCLUSION

The results demonstrated that kaempferol could prevent the growth of MRSA, as well as their ability to form biofilms, when treating MRSA by reducing viable populations of the bacteria and down-regulating expression of adhesin genes. In addition, evidence has been found that kaempferol may have a positive interaction (synergy or additive effect) with metformin; further studies are necessary to confirm this finding. The use of non-lethal pathways (i.e., biofilms, adhesins) to cure chronic infections should also be explored. Ongoing studies to understand kaempferol's mechanism of action and potential clinical utilization may lead to future developments for alternative approaches to eradicate MRSA infections and ultimately improve patient outcomes.

REFERENCES

1) Nazli, A., et al., Treatment of MRSA infection: where are we? Current medicinal chemistry, 2024. **31**(28): p. 4425–4460.
2) Kaushik, A., et al., Biofilm producing methicillin-resistant Staphylococcus aureus (MRSA) infections in humans: Clinical implications and management. Pathogens, 2024. **13**(1): p. 76.
3) Silva, V., et al., Biofilm formation of multidrug-resistant MRSA strains isolated from different types of human infections. Pathogens, 2021. **10**(8): p. 970.
4) Patel, H. and S. Rawat, A genetic regulatory see-saw of biofilm and virulence in MRSA pathogenesis. Frontiers in microbiology, 2023. **14**: p. 1204428.
5) Zhou, H., et al., The antibacterial activity of kaempferol combined with colistin against colistin-resistant gram-negative bacteria. Microbiology Spectrum, 2022. **10**(6): p. e02265–22.
6) Periferakis, A., et al., Kaempferol: antimicrobial properties, sources, clinical, and traditional applications. International journal of molecular sciences, 2022. **23**(23): p. 15054.
7) Hairil Anuar, A.H., et al., Critical evaluation of green synthesized silver nanoparticles-kaempferol for antibacterial activity against methicillin-resistant Staphylococcus aureus. International Journal of Nanomedicine, 2024: p. 1339–1350.
8) Deng, S.-P., et al., Facile synthesis of long-term stable silver nanoparticles by kaempferol and their enhanced antibacterial activity against Escherichia coli and Staphylococcus aureus. Journal of Inorganic and Organometallic Polymers and Materials, 2021. **31**(7): p. 2766–2778.
9) Majumdar, G. and S. Mandal, Antibacterial activity analysis of kaempferol and its derivatives targeting virulence and quorum sensing associated proteins by in silico methods. The Microbe, 2025. **6**: p. 100259.
10) Wang, L., et al., Linalool as a potential agent for inhibiting Escherichia coli biofilm formation and exopolysaccharide production. BMC Veterinary Research, 2025. **21**(1): p. 235.
11) Lahiri, D., et al., Antibiofilm and anti-quorum sensing activities of eugenol and linalool from Ocimum tenuiflorum against Pseudomonas aeruginosa biofilm. Journal of Applied Microbiology, 2021. **131**(6): p. 2821–2837.
12) Hsu, C.-C., et al., The inhibitory activity of linalool against the filamentous growth and biofilm formation in Candida albicans. Medical Mycology, 2013. **51**(5): p. 473–482.
13) Manoharan, R.K., et al., Inhibitory effects of the essential oils α-longipinene and linalool on biofilm formation and hyphal growth of Candida albicans. Biofouling, 2017. **33**(2): p. 143–155.
14) Rocha, F.R., et al., Effect of bioactive compounds on the regulation of quorum sensing network-associated genes and virulence in Streptococcus mutans—A systematic review. Archives of Oral Biology, 2020. **119**: p. 104893.
15) Cheruvanachari, P., et al., Deciphering the antibiofilm potential of 2-Phenylethyl methyl ether (PEME), a bioactive compound of Kewda essential oil against Staphylococcus aureus. Microbial Pathogenesis, 2023. **179**: p. 106093.
16) Vieira, S.F., et al., Plant-derived bioactive compounds as key players in the modulation of immune-related conditions. Phytochemistry Reviews, 2025. **24**(1): p. 343–460.
17) Chadha, J., et al., Anti-virulence prospects of Metformin against Pseudomonas aeruginosa: A new dimension to a multifaceted drug. Microbial Pathogenesis, 2023. **183**: p. 106281.
18) Khayat, M.T., et al., Synergistic benefits: exploring the anti-virulence effects of metformin/vildagliptin antidiabetic combination against Pseudomonas aeruginosa via controlling quorum sensing systems. Biomedicines, 2023. **11**(5): p. 1442.
19) Barisch, C., J.C. Holthuis, and K. Cosentino, Membrane damage and repair: a thin line between life and death. Biological chemistry, 2023. **404**(5): p. 467–490.
20) Murínová, S. and K. Dercová, Response mechanisms of bacterial degraders to environmental contaminants on the level of cell walls and cytoplasmic membrane. International journal of microbiology, 2014. **2014**(1): p. 873081.
21) Dalbey, R.E., P. Wang, and J.M. van Dijl, Membrane proteases in the bacterial protein secretion and quality control pathway. Microbiology and molecular biology reviews, 2012. **76**(2): p. 311–330.

22) Mitchell, A.M. and T.J. Silhavy, Envelope stress responses: balancing damage repair and toxicity. Nature Reviews Microbiology, 2019. **17**(7): p. 417–428.
23) Abbas, H.A., A.M. Elsherbini, and M.A. Shaldam, Repurposing metformin as a quorum sensing inhibitor in Pseudomonas aeruginosa. Afr Health Sci, 2017. **17**(3): p. 808–819.
24) Hegazy, W.A.H., et al., Repurposing Anti-diabetic Drugs to Cripple Quorum Sensing in Pseudomonas aeruginosa. Microorganisms, 2020. **8**(9).
25) Khayat, M.T., et al., Synergistic Benefits: Exploring the Anti-Virulence Effects of Metformin/Vildagliptin Antidiabetic Combination against Pseudomonas aeruginosa via Controlling Quorum Sensing Systems. Biomedicines, 2023. **11**(5).
26) S, M.S., et al., Crippling of Klebsiella pneumoniae virulence by metformin, N-acetylcysteine and secnidazole. BMC Microbiol, 2023. **23**(1): p. 229.