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Investigation of Silver and Copper Quantum Dots Prepared by Bio-Based Methods on Biofilm-Forming Genes in Gram-Positive and Gram-Negative Bacteria

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Abstract

The increase of pathogenic multi-drug resistive (MDR) bacteria has become one of the major threats of healthcare sector. This has further added to the urge for the development of novel drugs and formulations in combat against resistive pathogenic bacteria. The aim of this research is to examine the effect of copper and silver quantum dots prepared by a green method on the biofilm-forming genes of S. aureus and A. baumannii. The MIC of these QDs was determined. the expression level of biofilm-forming genes (icaA, icaD) in S. aureus and bap and OmpA in A. baumannii) with sub-MIC concentrations of copper and silver QDs was examined by Real-time PCR tests.The studied pathogenic bacteria included isolated from infections wounds. Moreover, S. aureus and A. baumannii species provided from microbial bank were also considered standard strains. Oak fruit was utilized for the green synthesis of QDs through a hydrothermal method. Gene expression studies statistically significant difference under the influence of silver and copper QDs compared to the gene expression of rRNA 16S. The findings revealed that Ag and Cu QDs declined the expression of biofilm-forming genes (ica A,D,R in S. aureus and BAP and OmpA in A. baumannii). Real-time PCR studies on the anti-biofilm impact of Ag and Cu QDs on S. aureus and A. baumannii showed a significant decrement (sometimes 5-fold decrease)in the expression of ica A,D,R and BAP and OmpA genes compared to the rRNA 16S reference.

Keywords: Biofilm, Gene expression, Metallic quantum dots, Pathogenic bacteria.

Introduction

The rise in the resistance of bacteria has endangered the health and life of human, necessitating the development of novel antibacterial compounds to fight against antibiotic-resistant microorganisms. To this end, quantum dots (QDs) have been considered whose antimicrobial activity is one their major characteristics. In contrary with conventional antibiotics, QDs offer high structural resistance in addition to robust structural stability (Seth et al. 2023).

Quantum dots have been emerged as multifunctional materials with organic, inorganic, and natural bases. They have revolutionized various biomedical applications especially our combat against pathogenic biofilms. QDs have offered promising solutions through physical mechanisms such as photothermal or photodynamic treatments to disturb the formation of biofilms. They have shown significant effectiveness compared to conventional antibiotics at lower resistance and on a wide range of species. The stability and durability of QDs guarantee the antibiofilm activities even under challenging conditions. This study comprehensively addresses the synthesis, properties, and application of QDs and displays pioneering advancements of these nanomaterials in innovations and research. These nanomaterials offer multifaceted mechanisms such as disturbance if the cell wall and membranes,

production of reactive oxygen species (ROS), and attachment to the nucleus substances to effectively prevent the proliferation of bacteria. Therefore, this research is aimed to evaluate the QDs as a potential factor in preventing pathogenic biofilms with emphasis on their promising potential (Koul et al. 2024).

World health organization (WHO) has recognized *S. aureus* as a high-priority pathogen requiring new treatment methods. This research presents novel treatments methods and nano-systems as a promising toll for the treatment of infections related to the *S. aureus* biofilm (Tacconelli et al. 2018).

The pathogenicity of polysaccharide biofilm layer of *S. aureus* is of crucial significance. In 2007, healthcare infection practices advisory control (HICPAC) presented the methods to fight against this pathogenic factor including biofilm through a special instruction (Thilakavathy et al. 2015). The presence of this layer is coded by a specific group of structural operons called intercellular adhesion (ICA) processing various gene loci such as ica A, icaD (Thilakavathy et al. 2015; Tyner and Patel 2016). These genetic loci are in close relationship with polysaccharide intercellular adhesion (PIA). icaA codes the enzyme in charge of polysaccharide biosynthesis while icaB codes another enzyme for polysaccharide deacetylation before its attachment on the cell surface (Namvar et al. 2013). The product is a protein passing through the surface of the membrane layers with homology with N-acetyl- glucosaminyltransferases. This gene is a primary inducer of the biofilm formation in *S. aureus* which causes cascade expression of ica locus by receiving environmental signals by PIAs (O'Gara 2007). This gene starts its activity in the presence of UDP-Nacetylglucosamine and is the only ica gene with transferase properties. icaD is a signal transmitter (chaperon) to other genes of this locus with activates specific enzymes for communication and expression of icaC, icaR, and icaD genes with the help of icaR. The biofilm expression showed a 23-fold increase upon the cooperation between icaR and icaD (Costa et al. 2013). This sometimes results in the production of biofilms in some strains even in the presence of icaDR. icaC is in charge of communication between the internal and external cytoplasm spaces of the bacteria and has the longest intermembrane sequences. This section causes communication of icaD with the external part of cytoplasm membrane as well as its relationship with icaB. icaB is the only locus situated outside of cytoplasm which maintains the surface relationship of bacteria with PIAs (Costa et al. 2013).

Multidrug-resistive (MDR) *A. baumannii* is an opportunistic pathogen involved in serious healthcare-associated infections (HAI). It can be assigned to various factors including the ability of this bacterium to form antimicrobial resistive (AMR) biofilms in addition to promoting the horizontal transformation of antibiotic-resistance genes (Abd El-Rahman et al. 2023).

The first member of biofilm-forming and biofilm-reinforcing proteins, called BAPs, was identified in *A. baumannii*. This protein can be found on the external surface of the bacterium. The central nucleon is composed on successive repetitions of similar sequences which enables the formation of biofilm, playing a significant role in the infection caused by these pathogens (Brossard and Campagnari 2012).

Proteins of the external membrane, especially OmpA, are the other pathogenic factor of *A. baumannii*. OmpA is a three-section protein with the weight of 38 kD, playing decisive role in the formation of biofilm on living and non-living surfaces through cell-cell, and cell-surface adhesion (McConnell et al. 2013).

The aim of this research is to investigate the influence of silver nanoparticles on the expression of BAP and OmpA genes which play pivotal role in biofilm formation in the drug-ressitive strains of *A. baumannii*.

The present research also addressed the antimicrobial effects of carbon QDs synthesized from plant sources.

Materials and methods

Bacterial strains, chemicals, and growth conditions

The pathogenic bacteria in this study included *S. aureus* (N=46) and *A. baumannii* (N=34) which were isolated from infection of diabetic, burning, and surgery wounds of patients in Razi Hospital from June to December 2023. The samples were assessed by common biochemical and microbiological tests such as catalase, indole, and citrate tests. *Acinetobacter baumannii* (PTCC 1797) and *S. aureus* (ATCC 29213) were also considered standard strains.

Synthesis of the QDs, MIC and sub-MIC determination of S. aureus and A. baumannii

Synthesis of carbon QDs

Oak fruit was utilized for the green synthesis of silver and copper QDs by hydrothermal method for the firms time. The oak fruits were first washed by deionized water and acetone, they were then dried at ambient conditions and powders by a mill. After optimization, 5 g of the powdered sample was added to 100 mL deionized water and heated up to boiling under vigorous stirring for 30 min. The resulting solution was centrifuged (5000 rpm) for 15 min giving rise to a biphasic product. The supernatant was filtered by a Wattman filter paper (No.1). the resulting solution (2mL) was placed in the TLC-UV chamber and the formation of QDs was confirmed by green fluorescent reflection. the remaining solution was heated to 190 ℃ in an autoclave for 5 h. after cooling at room temperature, the yellow suspension particles were heated in an oven at 80 for 2 h, resulting in crystalline brown powder. The powders were kept at 4 . Above figure shows the preparation method the carbon QDs.

Synthesis of silver QDs

The silver QDs were synthesized by adding about 20 mL of AgNO3 solution (1mM) to 20 mL of colloidal solution (0.01 mg/mL) under stirring (at the rate of one drop per second) such that the reductive factors reduced the Ag ions to achieve silver QDs. After adding all AgNO3 solution, the stirrer was turned off and the solution was kept at darkness to avoid any photochemical reversion. The color of the solution turned from reddish brown into yellowish brown after 1 h. the absorption spectra of the silver QDs were then recorded at 30 ℃ using a UV-Vis spectrometer (Figs. 1-3).

The solution of Ag QDs was then purified by centrifugation at 10000 rpm for 10 min followed by drying in an oven at 60 ℃ for 24 h to achieve solid QDs.

Synthesis of copper QDs

Freshly prepared copper sulfate solution (20 mL; 0.0.1M) was added to 20 mL oak fruit solution while stirring. The resulting mixture was magnetically stirred for 20 min at 70 ℃. Sodium hydroxide solution (1M) was then dropwise added to reach pH of 12. The solution was stirred for 2 h. The pH of the solution was about 3 before addition of sodium hydroxide. The gradual addition of NaOH changed the solution color from blue to green, dark green, and finally dark brown, suggesting the formation of copper oxide QDs. Fine brown particles were obtained after 2 h. the solution was then centrifuged for 10 min at 12000 rpm twice to remove all impurities. The synthesized particles were then dried in an oven for 24 h at 60 ℃.

Stability of QDs

UV-Vis spectroscopy

UV-Vis spectroscopy (Agilent; US 200-700 nm) was utilized to confirm the structure of the synthesized QDs 120 min after the reaction and color change.

Transmission electron microscopy

A transmission electron microscope (TEM; Zeiss EM10C) was also employed to assess the morphology and size of the QDs.

Determination od minimum inhibition (MIC) and minimum bactericidal concentration (MBC)

Microdilution broth technique based on CLSI instruction was utilized to determine MIC and MBC values (Jorgensen 2010). The bacterial strains were first separately cultured in MHB culture medium for 24 h at 37 ℃ and 120 rpm. In the next step, 50 µL of serial dilutions of silver and copper nanoparticles in MHB medium (50, 100, 200, 300, and 400 µg/mL) were added to 96-well microplate. Then, 5 µL of suspension at standard concentration (0.5 McFarland) was added to the wells and incubated at 37 ℃ for 24 h. one well containing culture medium and bacteria but with no nanoparticle was considered as the control. After incubation MIC and sub-MIC levels of each strain was determined as the lowest contrition at which no bacterial growth was observed.

Biofilm formation and antibiofilm activities of QDs

Biofilm formation was evaluated by the microtiter plate method of Lee et al. (2011), involving optical absorption measurement. The test was performed in triplicates for each strain.

Bacteria were classified into four groups based on their ability to form biofilm;

No biofilm production (OD ≤ ODc)

Poor biofilm producer (ODc 4 ≤ ODc <OD2)

Moderate biofilm producer (ODc 2 ≤ ODc <OD)

Strong biofilm producer (ODc <OD4).

Five isolates of S. aureus and five isolates of A. baumannii, as strong biofilm producer, were used to study the effect of quantum dots.

Total RNA extraction

In the present study, RNA was extracted from Acinetobacter baumannii and Staphylococcus aureus to examine the expression of bap, OmpA, and ica A, D, R genes after treatment with sub MIC concentration of metal quantum dots. RNA extraction was performed according to the instructions by Kiagen company. Briefly, a single colony was selected from each strain and inoculated into 5 mL of LB broth followed by incubation at 37°C for 24 h. Broth cultures (100 µL) were inoculated into 10 mL of fresh LB broth and grown to mid-phase (optical density of~ 0.5 at 600 nm) at 37°C on a shaker at 185 rpm. Finally, total RNA was extracted using the kit (Valadbeigi et al. 2023).

In the next step, cDNA synthesis was achieved by Takara (Japan) kit reagent RT™ Script Prime (Takara). For this purpose, 500 ng of the total extracted RNA was taken and cDNA synthesis was performed based on the instruction of the kit.

Real-Time PCR assessment of the expression of the biofilm producer genes

In this research, the master mix containing SYBER green (Biosystem Applied (England)) was used for the Real-Time PCR test. The primers of this research were designed by Primer Express and Runner Gene software. Then, the primers were confirmed using the sequence in the gee bank and online BLAST tool. The difference between the expression of the target genes of the control and treated samples was determined using Tukey's HSD post hoc test.

The expression level of OmpA, bap and ica A, D, R genes was measured as the ratio of expression of the target gene to that of the reference gene by relative quantification.

Table 1 shows the sequence of forward and reverse primer pairs of bap, OmpA, and ica A, D, R genes.

Table 1. Sequence of oligonucleotide primers used for RealTime PCR.

Gene		**Primer sequence (3' – 5')**	**Amplicon size (bp)**
ica A	F	TCTCTTGCAGGAGCAATCAA	188
	R	TCAGGCACTAACATCCAGCA	
ica D	F	ATGGTCAAGCCCAGACAGAG	225
	R	CGTGTTTTCAACATTTAATGCAA	
16srRNA S. aureus	F	AACCTACCTATAAGACTGGG	570
	R	CATTTCACCGCTACACATGG	

Bap	F R	TGAAAGTGGCTGCCAGTGAT TCTGCGTCAGCGTCACTATC	223
OmpA	F R	GCTGGTGTTGGTGCTTTCTG TCGGTTGATCCCAAGCGAAA	490
16srRNA A. baumannii	F R	TATCAGGACCATCTGGAGTAGG CATCAACTTCACCTTCACGC	110

RNA extraction was performed according to the instructions at the RNA concentration of about 1 to 2 µg. For this purpose, its optical absorption was measured at 260 nm. Moreover, optical absorption was measured at 280 nm to ensure the absence of protein contamination while absorption at 230 nm was assessed to ensure the absence of salt and other organic substances. For RNA, this absorption ratio was acceptable.

According to the instructions, master mix, primers, and synthesized cDNA were mixed in an appropriate volume to perform Real-time PCR reaction (Bioneerexieycler 96). The reaction volume was 25 microliters including 2 µL of cDNA, 10 pM of forward and reverse primers of ica, ADR, bap, and mapa genes, 1 µL of Bioneer master mix, and 11 µL of sterile double distilled water. Thermal cycling involved 10 minutes of initial denaturation at 95°C, followed by 40 cycles of 95°C for 20 s, and 58°C for 40 s. The final step to plot the melting curve was performed at 95°C for 15 s, 60°C for 30 s and 95°C for 15 s. For each sample, the reaction was performed twice and the average values were taken (quantity) as gene expression of that sample.

Statistical analysis

Statistical analysis was achieved using SPSS software package SPPSS Inc; for Windows version 18. The results were compared by one-way ANOVA with a probability below 0.05.

Results

Biofilm production

Overall, 36 *S. aureus* and 34 *A. baumannii* isolates were evaluated and 5 isolates of each bacterium were selected due to the production of strong biofilm. Isolates producing weak or moderate biofilms were excluded

Table 2. Biofilm production ability of S. aureus isolates.

Sample	**Biofilm**			
	WeaK	Moderat	Strong	Total
diabetic ulcer	٪12 (n = 2)	٪61 (n = 11)	٪27 (n = 5)	18
burn wound	٪10 (n = 1)	٪60 (n = 6)	٪30 (n = 3)	10
surgical wound	٪13 (n = 1)	٪50 (n = 4)	٪37 (n = 3)	8

Table 3. Biofilm production ability of A. baumannii isolates.

Sample	**Biofilm**			
	WeaK	Moderat	Strong	Total
diabetic ulcer	٪22 (n = 4)	٪42 (n = 8)	٪36 (n = 7)	19
burn wound	٪12 (n = 1)	٪55 (n = 5)	٪33 (n = 3)	9
surgical wound	٪33 (n = 2)	٪50 (n = 3)	٪17 (n = 1)	6

Determination of the MIC and Sub-MIC of silver and copper QDs by microdilution method

Five isolates capable of forming strong biofilms were selected from each bacterium. The microdilution results indicated that MIC levels of silver QDs were 300 and of copper 400 µg/mL, respectively. Moreover, sub-MIC of

silver and copper QDs (200 and 300 µg/mL, respectively) was considered to evaluate the anti-biofilm activities of the QDs.

Anti-biofilm activities of silver and copper QDs against biofilm-producing S. aureus and A. baumannii

Antibiofilm activity of nanoparticles was assessed against five isolates of each bacterium. They managed to inhibit biofilm-producing bacteria at concentrations Above 300 µg/mL of silver and concentrations Above 400 µg/mL of copper QDs. Therefore, all isolates were inhibited by the mentioned QDs at the OD level (0.22). Reference strains of *S. aureus* and *A. baumannii* were used as positive control

Effect of sub-MIC concentrations of silver and copper QDs on the expression of biofilm-producing genes

Biofilm-producing isolates of *S. aureus* and *A. baumannii* which were inhibited at sub-MIC concentrations of silver and copper QDs were selected for the present work. The Real-time PCR results indicated the similar expression of biofilm-producing genes with no significant difference. The expression of OmpA, bap, and ica ADR significantly (P-value<0.05) decremented upon exposure to sub-MIC levels of QDs (Fig. 1).

Real-time PCR investigation of expression of ica A,D genes in S. aureus under the influence of Ag QDs

Figs. 1-6 shows the expression ratio of the reference gene in S. aureus. The results indicated a statistically significant decrease in the expression of the mentioned genes under the influence of QDs. Ag QDs exhibited a stronger decrementing effect on the studied genes compared to the rRNA 16S (P< 0.05).

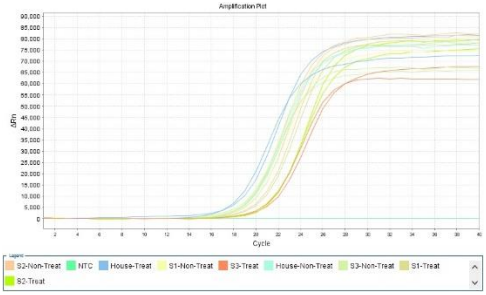

Fig. 1 Real-time PCR results for icaA gene in *S. aureus* isolates treated with QDAg

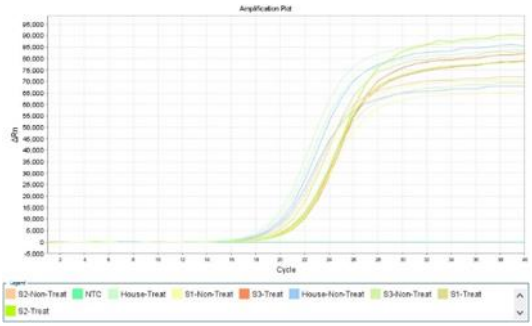

Fig. 2 Real-time PCR results for icaA gene in *S. aureus* isolates treated with QDCu

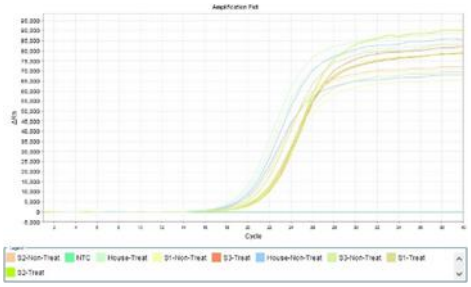

Fig. 3 Real-time PCR results for icaD gene in *S. aureus* isolates treated with QDAg

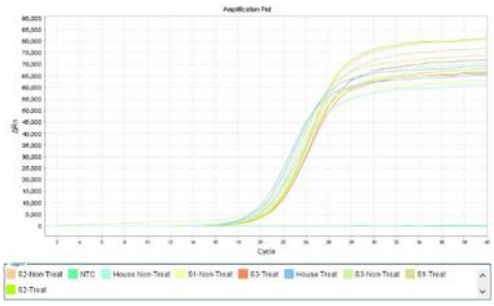

Fig. 4 Real-time PCR results for icaD gene in *S. aureus* isolates treated with QDCu

Real-time PCR investigation of expression of Bap and OmpA genes in A. baumannii under the influence of Ag QDs

This research addressed the impact of copper and silver QDs on expression of BAP and OmpA genes which are involved in the formation of biofilm in the drug-resistive A. *baumannii.* Figs. 7-10 displays the gene expression ratio of the reference genes in the mentioned bacterium. The results indicated a decline in the expression of OmpA and BAP genes under the influence of the two mentioned QDs where silver QDs showed stronger decremental effect with statistically significant difference compared to rRNA 16S ($p<0.05$).

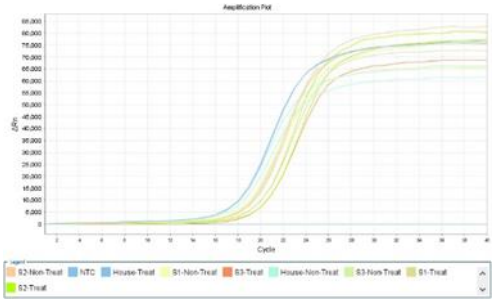

Fig. 7 Real-time PCR results for bap gene in *A. baumannii* isolates treated with QDAg

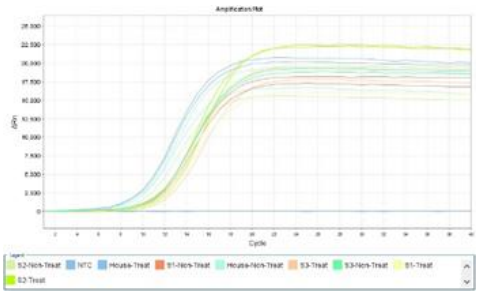

Fig. 8 Real-time PCR results for bap gene in *A. baumannii* isolates treated with QDCu

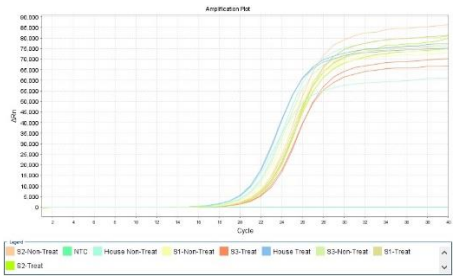

Fig. 9 Real-time PCR results for ompA gene in *A. baumannii* isolates treated with QDAg

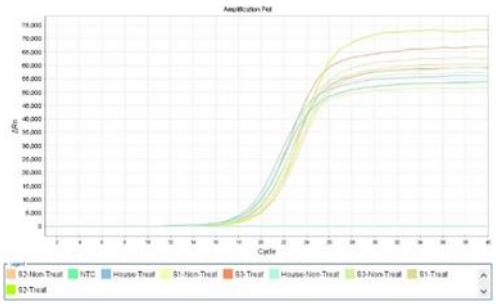

Fig. 10 Real-time PCR results for ompA gene in *A. baumannii* isolates treated with QDCu

Discussion

Humans have faced increasing challenge of antibiotic-resistant bacteria which has enhanced the mortality rate and the emergence of diseases with difficult management. Acinetobacter *baumannii* is one of these flexible pathogens whose rising prevalence in the hospitals and drug resistance can be assigned to increasing mortality rate. Despite its clinical significance, this pathogen has been poorly understood in non-hospital environments (Ahuatzin-Flores et al. 2024).

Moreover, S. aureus is a human pathogen, known as the main cause of infection in the healthcare sector. It can diversify reckless infections mainly due to developing resistance against several drugs. Its diverse pathogenic factors can develop biofilms on medical devices. Such chronic biofilm-related infections increase the mortality rate, causing high economic-social burden especially in the developing countries.

Biofilm is one of vital factors in the emergence of multi-drug resistance. High mortality rate and complications and damages related to the medical devices are assigned to the formation of biofilm which primarily require alternative treatment. Nanotechnology has recently emerged as a novel method to treat biofilm-related issues. The application of nanoparticles (NPs) in research works has gained considerable attention, resulting in rapid boost of research works in this field (Al-Wrafy et al. 2022).

Numerous review articles have comprehensively addressed various aspects of nanotechnology-based antibiofilm strategies. Wu et al. (2023) examined the latest nanotechnology-based approaches to disturb biofilm growth cycle. The authors studies current nano-based approaches to interfere the formation of bacterial biofilm in different stages, emphasizing the importance of biofilm regulation.

While several comprehensive review works have addressed nanotechnology-based antibiofilm strategies, our study specifically focuses on antibiofilm and antimicrobial activities of a range of nanoparticles including metallic quantum dots.

Green synthesis is an environmentally compatible technique for the synthesis of nanoparticles with a wide range of unique properties depending on the applied plant extract (Hosnedlova et al. 2022). In this research, silver and copper QDs with antibacterial and antibiofilm activities were prepared through a green synthesis route using oak fruit extract.

In the present work, a combination of S. aureus and A. *baumannii* biofilm was isolated from the mucus and infected wounds of patients in university-affiliated hospitals. Then, the effect of silver and copper QDs was addressed on the genes involved in the formation of biofilms of these bacteria.

QDs were used in sub-MIC level. The results indicated that silver and copper QDs have antibiotic activities on biofilm-producing S. aureus and A. baumannii bacteria at sub-MIC concentrations and higher.

Pourkhosravani et al. (2021) reported the influence of silver nanoparticles against S. aureus at the concentration of 300 μg/mL.

A series of bacteria including E. coli, Bacillus subtilis and Enterococcus faecalis have been tested for antibacterial and antibiofilm properties using nanoparticles (Asadi et al. 2023; Wang et al. 2021). However, this study for the

first time addressed the effect of silver and copper QDs on genes related to biofilm formation (ica A, D, R in S. aureus, as well as BAP and ompA in A. baumannii).

Similar to the current studies, the results of Afrasiabi and Partoazar (2024) research determined that nanoparticle (NP)-based treatment is one of the promising approaches against biofilms as nanoparticles inhibit the resistance of bacterial cells in planktonic forms through multiple mechanisms. For example, NPs such as silver (Ag), copper oxide (Cu), and iron oxide (Fe3O4) interfere with the gene expression of biofilm-associated bacteria through various strategies. NPs can penetrate the biofilm structure and affect the expression of efflux pump, quorum sensing, and adhesion-related genes, leading to the inhibition of biofilm formation or development (Afrasiabi and Partoazar 2024).

Gheidar et al. (2018) studied the expression of genes involved in biofilm formation in Staphylococcus aureus, including icaA, icaD, fnbA and fnbB to analyze the physiological response to controlled concentrations of such nanoparticles using RT-qPCR evaluations. The results of our study showed that nanoparticles are highly effective on antibiotic-resistant isolates and these compounds can be used in the treatment of resistant bacteria. This study also confirms the promising potential of using nanoparticles as anti-biofilm agents.

The findings of this study indicated a correlation between the biofilm formation and gene expression.

Our results showed that silver and copper QDs strongly reduced the expression of ica A, B, R genes in S. aureus and the expression of bap and ompA genes in A. baumannii. Therefore, this study showed that biosynthesized quantum dots can reduce biofilm.

In a similar study, Rezania et al. (2022) reported a decline in the expression of bap, csuC and csuE genes in A. baumannii strains treated with the MIC concentration of silver nanoparticles as compared to the control groups.

Hetta et al. (2021) studied multi-drug resistant Acinetobacter baumannii and determined that silver nanoparticles reduced the expression of virulence genes and biofilm.

Conclusions

The findings of this study show that silver and copper QDs inhibit biofilm formation in S. aureus and A. baumannii and can be used as potential anti-biofilm agents. Furthermore, our results emphasized the effect of silver and copper quantum dots on biofilm formation and their association with the expression ratio of ica A, D, R genes in Staphylococcus aureus bacteria and Bap and OmpA genes in Acinetobacter baumannii. ica A, D, R genes in S. aureus, as well as BAP and ompA in A. baumannii, were suppressed after treatment with silver and copper quantum dots. More importantly, silver QDs showed stronger effect on preventing biofilm formation in both bacteria. Therefore, silver and copper quantum dots can effectively control the expression of ica ADR systems in S. aureus as well as BAP and ompA in A. baumannii and. Hence, they can be regarded as promising anti-biofilm agents. Other genes involved in biofilm formation in S. aureus and A. baumannii should be also investigated.

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Author Contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Yasman Sadat Nabipour] , [Arman Rostamzad] , [Ardeshir Hesampour] , [Maryam Tajabadi] and [Salman Ahmadi Asbchin]. The first draft of the manuscript was written by [Ardeshir Hesampour] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval: IR.ILAM.REC.1401.008

References

1. Abd El-Rahman OA, Rasslan F, Hassan SS, et al (2023) The RND efflux pump gene expression in the biofilm formation of acinetobacter baumannii. Antibiotics 12(2):419
2. Afrasiabi S, Partoazar A (2024) Targeting bacterial biofilm-related genes with nanoparticle-based strategies. Front Microbiol 15:1387114
3. Ahuatzin-Flores OE, Torres E, Chávez-Bravo E (2024) Acinetobacter baumannii, a Multidrug-Resistant Opportunistic Pathogen in New Habitats: A Systematic Review. Microorganisms 12(4):644
4. Al-Wrafy FA, Al-Gheethi AA, Ponnusamy SK, et al (2022) Nanoparticles approach to eradicate bacterial biofilm-related infections: A critical review. Chemosphere 288:132603
5. Asadi S, Nayeri-Fasaei B, Zahraei-Salehi T, et al (2023) Antibacterial and anti-biofilm properties of carvacrol alone and in combination with cefixime against Escherichia coli. BMC Microbiol 23:55
6. Brossard KA, Campagnari AA (2012) The Acinetobacter baumannii biofilm-associated protein plays a role in adherence to human epithelial cells. Infect Immun 80(1):228-233
7. Costa MOC, Beltrame CO, Ferreira FA, et al (2013) Complete genome sequence of a variant of the methicillin-resistant Staphylococcus aureus ST239 lineage, strain BMB9393, displaying superior ability to accumulate ica-independent biofilm. Genome Announc 1(4):e00576-13
8. Gheidar H, Haddadi A, Kalani BS, Amirmozafari N (2018) Nanoparticles impact the expression of the genes involved in biofilm formation in S. aureus, a model antimicrobial-resistant species. J Med Bacteriol 7(3-4):30-41
9. Hetta HF, Al-Kadmy IM, Khazaal SS, et al (2021) Antibiofilm and antivirulence potential of silver nanoparticles against multidrug-resistant Acinetobacter baumannii. Sci Rep 11(1):10751
10. Hosnedlova B, Kabanov D, Kepinska M, et al (2022) Effect of biosynthesized silver nanoparticles on bacterial biofilm changes in S. aureus and E. coli. Nanomaterials 12(13):2183
11. Jorgensen JH (2010) Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline. https://goums.ac.ir/files/deputy_treat/md_labs_ef39a/files/CLSI-M45ed3e-2018(1).pdf
12. Koul K, Jawanda IK, Soni T, et al (2024) Quantum dots: a next generation approach for pathogenic microbial biofilm inhibition; mechanistic insights, existing challenges, and future potential. Arch Microbiol 206(4):158
13. Lee B, Schjerling CK, Kirkby N, et al (2011) Mucoid Pseudomonas aeruginosa isolates maintain the biofilm formation capacity and the gene expression profiles during the chronic lung infection of CF patients. Apmis 119(4-5):263-274
14. Manandhar S, Singh A, Varma A, et al (2018) Biofilm producing clinical Staphylococcus aureus isolates augmented prevalence of antibiotic resistant cases in tertiary care hospitals of Nepal. Front Microbiol 9:2749
15. McConnell MJ, Actis L, Pachón J (2013) Acinetobacter baumannii: human infections, factors contributing to pathogenesis and animal models. FEMS Microbiol Rev 37(2):130-155
16. Namvar AE, Asghari B, Ezzatifar F, et al (2013) Detection of the intercellular adhesion gene cluster (ica) in clinical Staphylococcus aureus isolates. GMS Hyg Infect Control 8(1):Doc03
17. O'Gara JP (2007) Ica and beyond: biofilm mechanisms and regulation in Staphylococcus epidermidis and Staphylococcus aureus. FEMS Microbiol Lett 270(2):179-188
18. Pourkhosravani E, Dehghan Nayeri F, Mohammadi Bazargani M (2021) Decoding antibacterial and antibiofilm properties of cinnamon and cardamom essential oils: a combined molecular docking and experimental study. AMB Express 11:1-18
19. Rezania N, Rahmati P, Noorbakhsh F, Farhadyar N, Lotfali E (2022) Investigation the effects of silver nanoparticles and gold nanoparticles on expression of bap and csu genes in biofilm formation of Acinetobacter baumannii. Iran J Microbiol 14(4):510
20. Seth S, Rathinasabapathi P, Selvarajan E, et al (2023) Quantum dots as antibacterial agents. In: Ethiraj S, Rathinasabapathi P, Samuel M, Chandrasekar N (eds) Carbon and graphene quantum dots for biomedical applications. Elsevier

21. Tacconelli E, Carrara E, Savoldi A, et al (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 18(3):318-327
22. Thilakavathy P, Priyan RV, Jagatheeswari P, et al (2015) Evaluation of ica gene in comparison with phenotypic methods for detection of biofilm production by coagulase negative staphylococci in a tertiary care hospital. J Clin Diagn Res 9(8):DC16
23. Tyner H, Patel R (2016) Propionibacterium acnes biofilm–A sanctuary for Staphylococcus aureus? Anaerobe 40:63-67
24. Valadbeigi H, Sadeghifard N, Kaviar VH, et al (2023) Effect of ZnO nanoparticles on biofilm formation and gene expression of the toxin-antitoxin system in clinical isolates of Pseudomonas aeruginosa. Ann Clin Microbiol Antimicrob 22(1):89
25. Wang Z, Shen Y, Haapasalo M (2021) Antimicrobial and Antibiofilm Properties of Bioceramic Materials in Endodontics. Materials (Basel) 14(24):7594
26. Wu J, Zhang B, Lin N, Gao J (2023) Recent nanotechnology-based strategies for interfering with the life cycle of bacterial biofilms. Biomater Sci 11(5):1648-1664