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The Antibiofilm Efficacy of Gold Nanoparticles Against *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is highly adapted to hospital environments, causing persistent chronic infections due to its ability to form biofilms. In this work, the antibiofilm activity of AuNPs with a subMIC concentration of 9.34 µg/ml was investigated by the microtiter plate method against 80 clinical isolates of *A. baumannii*. The results revealed that the biofilm was significantly (P< 0.05) reduced by 48.2 – 82.1%.

Keywords: Antibiofilm**,** Gold nanoparticles, *Acinetobacter baumannii*

كفاءة جزيئات الذهب النانوية في تثبيط الغشاء الحياتي لبكتريا *Acinetobacter baumannii*

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الخلاصه

بكتريا *Acinetobacter baumannii* مكيفة بشكل عال على العيش في بيئة المستشفى مسببة إصابات مزمنة عصية بسبب قابليتها على تكوين الغشاء الحياتي. في هذه الدراسة قيمت فعالية جزيئات الذهب النانوية ضد الغشاء الحياتي لثمانين عزلة سريرية من *A. baumannii* عند تركيز 9.34 مايكروغرام/مل باستعمال طريقة طبق المعايرة الدقيقة وأظهرت النتائج ان الغشاء الحياتي قد ثبط معنويا (P< 0.05) بحوالي 48.2 الى 82.1%.

Introduction

Acinetobacter baumannii is widely present in natural environments and frequently accompanying aquatic environments as an opportunistic bacterial pathogen which progressively increased the nosocomial infections [1, 2]. Of interest, this bacterial species is responsible for an extensive array of infections [3, 4]. Nevertheless, *A. baumannii* possesses an outstanding ability to thrive under a wide spectrum of environmental conditions and to survive in hospital settings, a feature that exposes its capacity for long-term persistent on abiotic surfaces via resistance to dryness and disinfectants [5, 6].
Biofilms are complex communities of microbes being predominantly attached to solid surfacesand they are habitually surrounded by thick polysaccharide matrix [7, 8]. The ability of *A. baumannii* to produce biofilm was established on both biotic and abiotic surfaces, while it plays an essential role in causing nosocomial as well as recurrence infections [9, 10]. The importance of biofilm is generated from its ability to decline the penetration of antibiotics, eventually initiating drug resistance [11-14]. It

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is well reported that biofilms are remarkably difficult to be eliminated and are frequently resistant to systemic antimicrobial treatment [15].
Such resistance has urged new approaches for biofilm treatment, one of which is employing gold nanoparticles (AuNPs) [16]. Giving that, the AuNPs display bacteriostatic or bactericidal activity against multi-drug resistant (MDR) pathogens [17].

Materials and Methods

Gold nanoparticles

In the present study the ecofriendly synthesized gold nanoparticles (particle size ranged 15 to 30 nm) were obtained from the Department of Biology, College of Science, University of Anbar.

Isolation and identification of *Acinetobacter baumannii*

A total of 260 clinical specimens were collected from wounds, burns, CSF, sputum and urinary tract infections. These specimens were obtained from patients referring to Baghdad Medical City hosptitals (viz. Gazi Al Harery Hospital, Burns Hospital, and Teaching Laboratories) and Ramadi Educational Hospital.

Specimens were cultured on MacConkey agar and incubated at 37°C for 24 hours. Afterward, the developed colonies were identified with the Vitek-2 compact system (bioMérieux, France) using a Gram Negative (GN) card according to the manufacturer's instructions.

Estimation of minimum inhibitory concentration of gold nanoparticles

The minimum inhibitory concentration (MIC) of GNPs was determined by the microdilution broth method. Briefly, serial concentrations of GNPs (150, 75, 37.5, 18.75, 9.37 and 4.68 μg/ml) were prepared with tryptic soy broth. The lowest concentration that inhibits the growth of bacteria is considered as the MIC.

Biofilm formation

The ability of *A. baumannii* to form biofilm was assayed by the microtiter plate method described by Cadavid and Echeverri [18] with some modification. In brief, an aliquot (20 μl) of bacterial suspension obtained from an overnight culture (comparable to McFarland standard No. 0.5) was used to inoculate microtiter wells containing 180 μl of Tryptone soya broth. Wells containing 200 μl of sterile Tryptone soya broth were considered as control. All microtiter plates were incubated at 37°C for 24 hr. Thereafter, the wells were washed thrice with phosphate buffered saline (pH 7.2) and dried at room temperature. A volume (200 μl) of methanol was added to the wells for 15 min. Afterwards, the plates were dried at room temperature. An amount (200 μl) of 0.1% crystal violet was added to the wells for 15 min. After that, the crystal violet solution was removed and washed thrice with phosphate buffered saline (pH 7.2) to remove the unbound dye. Drying the wells was accomplished at room temperature for 15 min. About 200 μl of 33% glacial acetic acid was added and the absorbance of each well was measured at 600 nm using a microplate reader (Biotek ELX 800, UK). Cut off value was calculated as the mean of OD_{600} of control plus 3 standard deviations. Any isolate with OD above cut off value was considered as biofilm producer.

Effects of gold nanoparticles on biofilm formation

The method described by Cadavid and Echeverri [18] was adopted to investigate the effect of AuNPs on biofilm formation. In brief, an overnight bacterial culture (in trypton soya broth) was adjusted to be compatible to with McFarland standard No. 0.5. Tryptone soya broth containing sub-MIC of AuNPs was inoculated with a previously prepared bacterial suspension and incubated for 24 hours at 37°C. An amount of 200 μl of the culture was transferred in triplicate into the vertical rows of a polystyrene microtiter plate well for each isolate and served as control. A volume of 200 μl of culture containing the sub-MIC concentration of the AuNPs was transferred into another three wells. All plates were incubated at a temperature of 37°C for 24 hours. Subsequently, the biofilm formation protocol was followed as mentioned earlier. Percentage of biofilm inhibition was calculated following the equation:

% of inhibition of biofilm formation = 1 – (O.D of treatment / O.D of control) × 100 …(1)

Statistical analysis

T test was employed to evaluate the biofilm inhibition activity of AuNPs using Excel application version 2019 from Microsft corporation.

Results and Discussion

Identification of *Acinetobacter baumannii* isolates

Out of 260 clinical specimens, 80 isolates were found to be *A. baumannii*; The microscopic examination showed Gram negative coccobacilli rods. These isolates were characterized as catalase-positive, indole and oxidase-negative, and non-fermentative of glucose. Generally, they appeared as smooth; yet, sometimes as mucoid colonies on solid media, with a colour ranging from white to pale yellow or greyish-white.

Estimation of minimum inhibitory concentration of gold nanoparticles

All the 80 *A. baumannii* isolates developed an MIC of 18.75 µg/ml; hence, sub MIC (9.37 µg/ml) was considered in subsequent experiments.

Antibiofilm Activity of AuNPs

The present work findings revealed that after 24 hr of treatment, the AuNPs significantly ($P< 0.05$) inhibited the biofilm formation (Table-1). The highest inhibition percentages of 80.9%, 80.4%, 82.1%, and 80.5% were accomplished by isolates M2, M19, M62, and M79, respectively. On the other hand, the lowest inhibition percentages of 23.5%, 26.5, 24.9%, and 25.2% were developed by isolates M28, M31, M48, M48 and M51, respectively. Moreover, other isolates revealed moderate inhibition range (48.2% - 72.5%). The current results showed that the highest inhibition percentage of biofilm formation after 24hr of treatment with sub- MIC (9.34 µg/ml) AuNPs was observed with the burn isolate M62 (82.1%), while the lowest inhibition percentage was developed by the wound isolate M28 (23.5%). Such variation in the inhibitory effect of AuNPs is more likely due to the difference in isolation source, environmental conditions that may cause changes in the isolates, as well as the difference in the physiological activity of each isolate due to the difference in their genetic structure, which in turn reflects their different metabolic and enzymatic activities.

Table 1-The inhibitory effects of Au-NPs on *Acinetobacter baumannii* biofilm formation

Isolate code	OD_{600}		Inhibition (%)	T test	Isolate code	OD_{600}		Inhibition (%)	T test
	Before treatment	After treatment				Before treatment	After treatment		
M1	0.629	0.263	58.2	3.3E-06	M41	0.285	0.106	62.9	2.6E-09
M2	0.658	0.126	80.9	8.0E-09	M42	0.270	0.102	62.7	1.2E-05
M3	0.745	0.286	61.7	1.3E-08	M43	0.304	0.091	70.1	1.3E-09
M4	0.393	0.162	58.8	5.0E-07	M44	0.245	0.085	65.5	4.1E-09
M5	0.804	0.388	51.8	8.9E-11	M45	0.295	0.090	69.4	7.9E-06
M6	0.408	0.202	50.5	6.9E-07	M46	0.548	0.199	63.7	2.7E-06
M7	0.422	0.219	48.2	1.6E-09	M47	0.565	0.219	61.3	1.9E-10
M8	0.687	0.254	63.1	7.6E-11	M48	0.323	0.242	24.9	1.0E-05
M9	0.498	0.205	58.9	3.9E-06	M49	0.285	0.080	71.9	2.7E-06
M10	0.273	0.104	62.0	3.3E-09	M50	0.305	0.099	67.7	2.3E-33
M11	0.587	0.206	65.0	1.1E-07	M51	0.438	0.328	25.2	1.8E-08
M12	0.526	0.225	57.3	1.1E-05	M52	0.445	0.219	50.8	1.0E-09
M13	0.417	0.202	51.6	2.0E-08	M53	0.435	0.165	62.2	5.0E-10
M14	0.827	0.412	50.2	1.4E-09	M54	0.318	0.098	69.1	1.1E-09
M15	0.453	0.190	58.1	1.9E-05	M55	0.295	0.083	71.9	1.3E-09
M16	0.241	0.105	56.5	7.8E-09	M56	0.518	0.196	62.1	2.5E-10
M17	0.407	0.197	51.6	6.6E-07	M57	0.423	0.207	51.0	1.2E-09
M18	0.322	0.122	62.2	2.7E-08	M58	0.300	0.094	68.8	7.9E-06
M19	0.464	0.091	80.4	2.4E-09	M59	0.295	0.088	70.1	1.5E-09
M20	0.267	0.110	58.9	5.4E-05	M60	0.460	0.133	71.1	3.1E-06
M21	0.276	0.110	60.2	4.8E-05	M61	0.630	0.263	58.2	2.5E-06
M22	0.271	0.101	62.8	3.1E-07	M62	0.658	0.118	82.1	3.1E-11
M23	0.281	0.090	68.0	2.7E-05	M63	0.745	0.286	61.7	6.0E-11

M24	0.246	0.085	65.5	1.3E-05	M64	0.394	0.162	58.8	9.2E-10
M25	0.294	0.090	69.4	3.2E-05	M65	0.805	0.388	51.8	8.8E-11
M26	0.547	0.199	63.7	2.8E-06	M66	0.428	0.200	53.3	6.4E-06
M27	0.565	0.219	61.3	1.5E-07	M67	0.453	0.209	53.9	5.6E-06
M28	0.320	0.245	23.5	5.9E-05	M68	0.685	0.254	63.1	7.7E-11
M29	0.290	0.080	72.5	1.5E-10	M69	0.499	0.205	58.9	1.8E-32
M30	0.306	0.099	67.7	3.1E-05	M70	0.274	0.124	54.8	5.3E-09
M31	0.435	0.320	26.5	2.5E-05	M71	0.588	0.205	65.0	1.2E-10
M32	0.444	0.209	53.0	4.7E-07	M72	0.526	0.226	57.3	3.3E-10
M33	0.436	0.165	62.2	4.5E-06	M73	0.416	0.203	51.6	1.3E-09
M34	0.317	0.098	69.1	7.0E-06	M74	0.825	0.412	50.1	9.2E-11
M35	0.295	0.083	71.9	7.4E-06	M75	0.453	0.190	58.1	4.8E-06
M36	0.517	0.196	62.1	1.3E-05	M76	0.241	0.105	56.5	7.8E-09
M37	0.422	0.207	51.0	6.1E-07	M77	0.407	0.197	51.6	7.6E-06
M38	0.301	0.094	68.8	1.5E-09	M78	0.322	0.122	62.2	8.3E-06
M39	0.294	0.088	70.1	6.9E-07	M79	0.464	0.091	80.5	1.4E-10
M40	0.460	0.133	71.1	3.1E-06	M80	0.267	0.110	58.9	5.4E-05

OD= Optical Density

The present results are in agreement with the previously reported anti-biofilm activity of AuNPs by AL-Taee [19], who showed that Au-NPs have high effectiveness in inhibiting the ability of MRSA biofilm formation, as detected by the microtiter plate assay that is considered as the best and most reliable method of detecting biofilm production and the adhesion of bacteria. Studies also found that both AgNPs and AuNPs of nanoparticles inhibited the biofilm formation by *Pseudomonas aeruginosa* and *E. coli* at the sub-MIC concentrations of 6.25 mg/ml for AgNPs and 12.5 mg/ml for the AuNPs. Biofilm inhibition at sub-MIC concentrations might be due to the non-lethal damage or the inhibitory effect on the expression of genes related to motility and biofilm formation [20]. A study carried out by Yu *et al.* [21] showed that the AuNPs exhibited antibiofilm activity on *P. aeruginosa*. AuNPs showed significant inhibitory effects only when their concentration reached 10 ppm (IC50 = 68.56–75.01 ppm). However, 5 ppm of AuNPs strongly inhibited biofilm formation (IC50 = 6.851–6.937 ppm). Hence, AuNPs also strongly attenuated biofilm formation of the pathogenic bacterium. The results of clinical specimens biofilm formation showed that the gold nanoparticles have different effectiveness in inhibiting the ability of *A. baumannii* biofilm formation. This could be due to the variance in isolation sites, variance in environmental conditions that may cause appearance changes in the isolates, and variance in the physiological activity of each isolate due to the variance in their genetic structure, which in turn reflects their different metabolic and enzymatic activities.

The mechanism of action of AuNPs that interacts with bacterial cells involves the release of ions by the nanoparticles, which interact with the thiol (- SH) group of transport proteins that emerge from the membrane of the bacterial cell. This could disrupt permeability and cellular respiration functions or interfere with system components of the electron transport chain in bacteria, leading to bacterial cell death [22].

Conclusion

This study demonstrated that the AuNPs, at concentration of 9.34 μg/ml, reduced the ability of *A. baumannii* to produce biofilm in a range of 60.8 – 80.9%.

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