Original article

Effect of silver nanoparticles on biofilm-forming *Pseudomonas aeruginosa* isolates from Menoufia University Hospitals

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A R T I C L E I N F O

A B S T R A C T

Background: Numerous infections can be caused by Pseudomonas aeruginosa, particularly in hospitalized patients. The aim of the study was to estimate the antibacterial and biofilm inhibition effects of Silver nanoparticles (AgNPs) on P.aeruginosa isolates. **Methodology**: A total of 283 samples were taken from hospitalized patients at Menoufia University Hospitals. P. aeruginosa isolates were identified by standard microbiological methods and VITEK 2 system. Antibiogram and biofilm formation were assessed by disk diffusion and modified congo red agar methods respectively. PslA and lasR genes were detected by conventional PCR. The antibacterial and anti-biofilm effects of AgNPs were also tested. **Results**: P. aeruginosa isolates represented 16.7% of isolates, 92% of them were resistant to piperacillin and ciprofloxacin. Resistance to aztreonam, piperacillin/tazobactam, cefepime, ceftazidime, and gentamicin were 74%, 72%, 72%, 62%, and 52% respectively. P. aeruginosa isolates were sensitive to meropenem (68%) and imipenem (60%). Approximately 54% and 44% of the isolates were ESβL and biofilm producers respectively. PslA and lasR genes were found among 52% and 48% of P. aeruginosa isolates respectively. The MIC and MBC of AgNPs ranged from (6.25 to 50 μg/mL), inhibiting 40.9% of biofilms at 6.25 μg/mL and further inhibiting 40.9% of biofilms at 12.5 μg/mL. **Conclusion**: P. aeruginosa are serious nosocomial pathogens due to high antimicrobial resistance that is mainly due to ESβLs production and biofilm formation. AgNPs exert potent antibacterial and anti-biofilm effects at concentrations of 6.25–50 μg/ml.

Introduction

P. aeruginosa causes 10–20% of all health-care associated infections (HAIs) including bacteremia, sepsis, UTI, pneumonia, burn and wound infections, particularly in patients admitted to intensive care units [1].

For hospitalized patients, multidrug-resistant *P. aeruginosa* is a serious public health issue. *P. aeruginosa* infections are challenging to

cure and are frequently accompanied by significant morbidity and mortality rates [2].

P. aeruginosa has the capacity to release many virulence factors (mucoid exopolysaccharides, lipopolysaccharides, biofilm, pili, exotoxin A, pigments, lipases, proteases, hemolysins, leukocidin, and rhamnolipids), which are implicated in colonization, invasion, and dissemination of the bacterium. These factors impair

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the host's immune system and create an antibiotic resistance barrier [3].

The pslA and lasR genes are involved in the synthesis and regulation of components of biofilm matrix including exopolysaccharides and extracellular DNA. The pslA gene encodes a glycosyltransferase enzyme that catalyzes the polymerization of Psl polysaccharide, which is important in initial adhesion and aggregation of *P. aeruginosa* cells on surfaces. The lasR gene encodes a transcriptional regulator in the quorum sensing system that controls the expression of numerous genes that mediate communication of bacterial cells and trigger synthesis of extracellular matrix relevant to biofilm formation [4, 5].

Biofilm-producing *P. aeruginosa* causes severe infections, especially in immunocompromised patients. These infections are difficult to be treated because extracellular matrix of biofilm is one of the main elements that reduce the penetration of antibiotics and the emergence of resistance [5].

Therefore, other therapeutic agents (non-antibiotic) alone or in combination with antibiotics would be quite effective in the treatment of infections with multi-drug resistant *P. aeruginosa*. These strategies include the usage of phage therapy, iron chelation, nanoparticles, vaccination, electrochemical scaffolds, and antimicrobial peptides, as well as quorum sensing and bacterial lectins inhibition [6].

Recently, nanoparticles have been viewed as a strong substitute for antibiotics and have a good chance of addressing the issue of bacterial multidrug resistance. In addition to penetrating bacterial cell walls and causing the bacterial cells to be destroyed by the production of free radicals, AgNPs can also produce silver ions that can form bonds with important organelles and cause those organelles to malfunction [2].

Some studies have considered that the fundamental mechanisms of AgNPs in biofilm eradication are their binding to the exopolysaccharide matrix, which disrupts biofilm integrity by identifying the peptidoglycan in the bacterial cell wall, leading to physical damage and also, electrostatic interactions between AgNPs and bacterial membranes lead to membrane rupture, allowing penetration of AgNPs through the mature biofilm [7].

Nowadays, Wound dressings, medical device coatings, and AgNPs-impregnated textile fabrics are further examples of AgNPs uses. The benefit of using AgNPs for impregnation is that silver ions are continuously released, and the devices could be coated on both the interior and exterior, enhancing their antibacterial efficacy. Burn injuries treated with AgNPs have better cosmoses and scar-free healing. More research is needed to establish the interactions between AgNPs and antibiotics [7].

The aim of this study was to assess the antibacterial and anti-biofilm activity of AgNPs on isolated *P. aeruginosa*.

Patients and Methods

Study design and patients:

This analytical cross-sectional study was conducted from January 2021 to August 2022. It included 283 clinical specimens (sputum, urine, pus, wound swab, blood and burn swabs) collected from patients at different departments (ICU, burn unit, surgery, urology, pediatrics and internal medicine), who developed symptoms and signs of HAIs which are infections affecting the patient as a result of admission and were not present at admission time. For the majority of patients, HAIs appear 48 hours or more after admission [9]. All the selected patients were subjected to detailed history taking (including exposure to invasive procedures like surgery, urinary catheters, central venous lines or endotracheal tubes) and comprehensive clinical examinations. The Menoufia University Local Ethics Committee (11/2020MICR-17) approved the study protocol. Before involvement in this study, written consent was taken from each patient or his/her legal guardians. Inclusion criteria included patients who had HAIs. Exclusion criteria were patients who refused participation in the study, those with good response to antibiotic therapy or were colonized without signs of infections.

Sample size calculation:

According to Karimipour and Tanomand, [8] with power 80% and confidence level 95%. Sample size was calculated and found to be 40 subjects.

Identification of *P. aeruginosa*

Samples were processed at Medical Microbiology and Immunology Department, Faculty of Medicine, Menoufia University. Identification of obtained colonies up to species level occurred via conventional techniques

including Gram stain, culture using various bacteriological media (nutrient agar, blood agar, macConkey's, sabouraud's dextrose agar, mannitol salt agar, and cetrimide agar) (Oxoid, UK) then incubated aerobically at 37°C for 24 hours, followed by biochemical reactions (TSI, urease, MIO, oxidase ,and catalase)(2). Identification was then confirmed using VITEK2 compact device system (Biomeriuex, France) using GN ID cards. Confirmed P. aeruginosa isolates were preserved at -80° in the suspension of the brain heart infusion with 20% glycerol for subsequent gene analysis by PCR.

Antimicrobial susceptibility test

Antimicrobial susceptibility for *P. aeruginosa* isolates was performed using Kirby-Bauer disk diffusion method against different antimicrobial agents (Oxoid, UK) and interpreted according to Clinical Laboratory Standard Institute 2022 CLSI [12]. The tested antimicrobial agents included piperacillin/tazobactam (TZP,100/10 μg), azetreonam (ATM,30 μg), cefepime (FEP, 30 μg), ceftazidime (CAZ,30 μg), piperacillin (PPL,100μg), imipenem (IPM,10 μg), meropenem (MEM,10 μg), levofloxacin (LEV,5 μg), ciprofloxacin (CIP,5 μg), ofloxacin (OFX,5 μg), gentamicin (CN,10 μg), tobramycin (TOB,10 μg), amikacin (AK,30 μg). Multidrug-resistant *P. aeruginosa* included isolates that were resistant to at least one agent in three or more antimicrobial groups, whereas extensively drug-resistant isolates were those that were susceptible to only one or two categories and pandrug-resistant *P. aeruginosa* included those with resistance to all agents in all antimicrobial categories [13].

Detection of ESβL production

Screening and phenotypic confirmation of ESβLs producers were performed using ceftazidime (30μg), cefotaxime (30 μg), and ceftriaxone (30μg). If the diameter of inhibition zone was less than 22 mm for ceftazidime, less than 27 mm for cefotaxime and less than 25 mm for ceftriaxone, the strain was regarded as suspicious for ESβL production. For confirmation, ceftazidime (30 μg) and ceftazidime/clavulanic acid (30/10 μg) discs were put 20 mm apart on MHA plate and incubated aerobically at 37°C for 18-24 hrs. ESβL producing isolate was considered when the diameter of the ceftazidime/clavulanic acid disk was 5 mm or more than that of ceftazidime disk alone [12, 14].

Phenotypic identification of biofilm development

Modified congo red agar (MCRA) was used. Inoculated agar was incubated at 37°C for 48 h and then for 2-4 days at room temperature. Dry black color was interpreted as strong positive biofilm, Weak biofilm producer gave occasional darkening only in the center while red colonies was interpreted as negative biofilm producers [5].

Genotypic detection of biofilm associated genes (pslA, lasR) by conventional PCR

DNA extraction

Pure *P. aeruginosa* colonies were obtained by culturing on cetrimide agar (HI MEDIA) after multiple streaking plate method. According to the instructions of the manufacturer, bacterial DNA was extracted and purified from 50 isolated *P. aeruginosa* using the QIAamp DNA Mini Kit (Germany) (QIAamp® DNA Mini and Blood Mini Handbook, Fifth Edition, 2016). Table 1 lists the primers that were used. After DNA extraction, its concentration and purity were evaluated by spectrophotometer using the Nanodrop (Implen, Germany). The ratio of the readings at 260 nm and 280 nm (A260 / A280) ranged from 1.7 to1.9.

PCR amplification

For lasR gene and pslA gene, the following components were added to a thin-walled 25-μl PCR tube: 12.5μlMyTaq PCRTM Master Mix (2x), 1 μl forward primer, 1 μl reverse primer, 0.5 μl Water nuclease–free and 10μl DNA Extract.

For the pslA gene amplification, initial denaturation at 94°C for 5 min, followed by 35 cycles of DNA denaturation for 30s at 94°C, primer annealing for 40s at 52°C and final extension for 50s at 72°C [15].

For the lasR gene, initial denaturation at 94°C for 5 min followed by 30 cycles of DNA denaturation at 94°C for 1m, annealing at 52°C for 1 min and final extension at 72°C for 1.5 min [16].

Electrophoresis was carried out on agarose gel 1.5% (EGY technology) stained with ethidium bromide (Sigma, USA) for 20 minutes. UV trans-illuminator was used to visualize the products using DNA molecular weight (MW) marker: (Ladder 50 bp ladder) (Bioline, UK) (code BIO-33056) for detection of pslA and lasR genes.

Testing of the anti-bacterial effect of AgNPs

NanoTech, Egypt provided a stock solution of 200 μg/ml spherical, water soluble AgNPs (20±5 nm). AgNPs were prepared by chemical reduction as reported by the manufacturer. Serial two-fold

dilutions were used to create different concentrations of AgNPs from the stock solution, ranging from 100 μg/ml to 3.125 μg/ml. The bacterial suspension turbidity was adjusted to 0.5 McFarland Standard. 100μl of each concentration and 100 μl of diluted cultures of each isolate were added to the wells of the 96-well micro dilution flat bottom plate, which had been labeled with different AgNPs concentrations. The lowest AgNPs concentration at which no visible growth can be seen was identified as minimal inhibitory concentration (MIC). After a further 24 hours of incubation, a minimal bactericidal concentration (MBC) was determined as the maximum dilution of AgNPs that still prevented bacteria growth on agar plates [17].

Assessment of the anti-biofilm effect of AgNPs

About 2 ml of sterile Trypticase soy broth was poured into labeled tubes. 50 μl of freshly cultured strain was then added to each test tube. Then AgNPs at two-fold dilutions from 100 μg/ml to 3.125 μg /ml were added. Then, each labeled individual MCRA was inoculated, and the mixture was incubated aerobically at 37 °C for 24 hours. The color of the colonies was checked and interpreted - as mentioned before-under the heading Phenotypic identification of biofilm development.

Statistical Analysis

Using SPSS version 23, data were gathered, tabulated and analyzed. The Chi-square (χ2) test was used for comparison of categorical variables. The p-value cut-off for statistical significance was at < 0.05.

Results

About 299 isolates were obtained from 283 clinical samples obtained from different hospital departments. The Gram-negative bacilli, Gram-positive cocci and Candida were identified in 74.6 %(223/299), 24.4% (73/299) and 1% (3/299) respectively. Out of 50 *P. aeruginosa* isolates (16.7%; 50/299), 30 isolates were obtained from burn unit (60%) followed by 12 isolates from ICUs (24%), 6 isolates from urology department (12%), and one from each of pediatric department and surgical department (2%) (Table-2).

P. aeruginosa infections were more common in patients aged above 60 years (30%), males (56%) and in all patients with invasive procedures

The majority of *P. aeruginosa* isolates were resistant to piperacillin (92%), ciprofloxacin (92%), levofloxacin (78%), ofloxacin (78%),

azetreonam (74%), cefepime (72%), and piperacillin-tazobactam (72%). On the other hand, *P. aeruginosa* isolates were sensitive to meropenem (68%) and imipenem (60%) (Table-3). About 44% of *P. aeruginosa* isolates were Multi-drug resistant, 12% were extensively-drug resistant and 6% were Pan-drug resistant.

ESβL production was detected among 31/50 (62%) by screening disk diffusion method and only 27/50 (54%) of them were confirmed by cephalosporin/clavulanate combined test with statistically significant difference (P-value <0.05). Except for tobramycin and ofloxacin, ESβLs producing *P. aeruginosa* showed higher rates of antibiotic resistance than non- ESβL producers. (Table-3).

Out of 50 *P. aeruginosa* isolates, 44% (22/50) were found to be biofilm producers using the MCRA method. Biofilm-forming isolates were 100% resistant to ceftazidime, 95.5% to ciprofloxacin, and 90.9% to piperacillin, aztreonam and ofloxacin. About 86.4% of biofilm-producing isolates were resistant to piperacillin/tazobactam and cefepime but lower resistance occurred with meropenem (50%), imipenem (59.1%) and gentamicin (68.2%). Biofilm production was detected among18/27(66.7%) of ESβL producing *P. aeruginosa*. Meanwhile, only 17.4% of non-ESβL were biofilm producers.

Out of *P. aeruginosa* isolates, 52% (26/50) and 48 % (24/50) were positive for the pslA and lasR genes respectively. About 36% (18/50) of isolates had both pslA and lasR genes (figure -1a and figure -1b).

Among biofilm producers, pslA and lasR genes were detected in 20/22(90.9%) and 18/22(81.8%) respectively (P-value <0.001), Among ESβL producing isolates, pslA and lasR positive isolates represented 16/27 (59.3%) and 14/27(51.9%) respectively with no statistically significant difference [Table -4].

All pslA positive *P. aeruginosa* isolates were resistant to quinolones group (ciprofloxacin, levofloxacin and ofloxacin), 92.3% (24/26) were resistant to each of piperacillin and ceftazidime, 88.5 % (23/26) were resistant to aztreonam and 80.8% (21/26) of isolates were resistant to gentamicin and piperacillin/tazobactam.

Among lasR positive *P. aeruginosa* isolates, 100% (24/24) were resistant to piperacillin, ceftazidime, ciprofloxacin, and levofloxacin, 95.8%

(23/24) were resistant to cefepime, aztreonam and piperacillin/tazobactam, 91.7% (22/24) were resistant to ofloxacin and 83.3% (20/24) were resistant to tobramycin.

Regarding the antibacterial effect of AgNPs, higher concentrations were needed for bacterial growth inhibition in biofilm-producing isolates. MIC in biofilm producers ranged from 6.25 to 25 µg/ml, of which 36.4% had MIC of 25 µg/ml. On the other hand, the range of MIC in non-biofilm producers was (6.25 - 25 µg/ml), and 75% of them had MIC of 6.25 µg/ml [Table-5].

The MIC of AgNPs was 6.25 µg/mL in 81.6% (14/19) of antibiotic-susceptible *P. aeruginosa isolates*, MIC for inhibition of 55% of antibiotic resistant strains ranged from 12.5 to 25µg/ml, with statistically significant difference (P<0.05). This result suggests that higher concentrations of AgNPs are required for inhibition of antibiotic-resistant strains.

Regarding anti-biofilm effect of AgNPs, the biofilm formation was inhibited in 40.9% (9/22) of isolates at concentration were 6.25 µg/mL of AgNPs, while 12.5 µg/mL of AgNPs inhibited formation of biofilm in 81.8% (18/22) and at concentration 25 µg/mL, biofilm formation was inhibited in all isolates [figures- 2a and figure -2b].

Table 1. Pimers sequences of pslA and lasR genes.

Gene	Primer sequence 5'——3'	Annealing temperature	Amplicon size (bp)	References
pslA	**Forword** TGGGTCTTCAAGTTCCGCTC **Reverse** ATGCTGGTCTTGCGGATGAA	52°C	119	*(Maita and Boonbumrung, (15)*
lasR	**Forword** AAGTGGAAAATTGGAGTGGAG **Reverse** GTAGTTGCCGACGACGATGAAG	52°C	130	*Lima et al., (16)*

Table 2. Distribution of the isolated organisms among different departments and ICUs (n=299)..

Isolated organisms	Total (n=299)	Burn unit	Surgery	Urology	ICU	Pediatrics	Internal medicine
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Staph aureus	62 (20.7%)	10 (16.1%)	2 (3.2%)	6 (9.7%)	40 (64.5%)	3 (1.0%)	1 (1.6%)
Coagulase-negative *Staphylococci*	6 (2.0%)	-	1 (16.7%)	1 (16.7%)	4 (66.7%)	-	-
Strept. pneumoniae	5 (1.7%)	-	-	-	4 (80%)	-	1 (20%)
Klebseilla spp.	84 (28.1%)	30 (35.7%)	2 (2.4%)	18 (21.4%)	32 (38%)	1 (1.2%)	1 (1.2%)
E. coli	69 (23.1%)	-	4 (5.8%)	34 (49.3%)	23 (33.3%)	3 (4.3%)	5 (7.2%)
Pseudomonas aeruginosa	50 (16.7%)	30 (60%)	1 (2%)	6 (12%)	12 (24%)	1 (2%)	-
Acinetobacter spp.	9 (3.0%)	-	1 (11.1%)	3 (33.3%)	4 (44.4%)	1 (11.1%)	-
Proteus spp.	5 (1.7%)	-	1 (20%)	1 (20%)	3 (60%)	-	-
Enterobacter spp.	5 (1.7%)	-	-	2 (40%)	3 (60%)	-	-
Citrobacter spp.	1 (0.3%)	-	-	1 (100%)	-	-	-
Candida spp.	3 (1.0%)	-	1 (33.3%)	1 (33.3%)	1 (33.3%)	-	-

Table 3. Antibiotic resistance of *Pseudomonas aeruginosa* in relation to ESβL production

| Resistant antimicrobial agents	Antimicrobial resistance	ESβL (No =27)	Non-ESβL (No =23)	X2	p
Piperacillin	**46 (92%)**	27 (58.7)	19 (41.3)	5.104*	0.038*
Piperacillin/tazobactam	**36 (72%)**	15 (41.7)	21 (58.3)	7.873*	0.005*
Ceftazidime	**31 (62%)**	27 (87.1)	4 (12.9)	35.975**	<0.001**
Cefepime	**36 (72%)**	27 (75.0)	9 (25.0)	22.826**	<0.001**
Aztreonam	**37 (74%)**	27 (73.0)	10 (27.0)	20.623**	<0.001**
Imipenem	**16 (32%)**	14 (87.5)	2 (12.5)	10.630*	0.001*
Meropenem	**13 (26%)**	13 (100)	0 (0.0)	14.965**	<0.001**
Gentamicin	**26 (52%)**	16 (61.5)	10 (38.5)	1.239	0.266
Tobramycin	**24 (48%)**	10 (41.7)	14 (58.3)	2.826	0.093
Amikacin	**24 (48%)**	10 (41.7)	14 (58.3)	2.826	0.093
Ciprofloxacin	**46 (92%)**	25 (54.3)	21 (45.7)	0.028	1.000
Levofloxacin	**39 (78%)**	24 (61.5)	15 (38.5)	4.056*	0.044*
Ofloxacin	**39 (78%)**	19 (48.7)	20 (51.3)	1.991	0.158

X2: **Chi square test**   p: p value for comparing between **ESβL** was detected by cephalosporin/clavulanate combined test and **Non-ESβL** in Pseudomonas

Table 4. prevalence of biofilm genes (pslA, lasR) among biofilm and ESβL producing *Pseudomonas aeruginosa* isolates.

Variable data	*PslA* gene				Total		*lasR* gene				Total	
	Positive		Negative				Positive		Negative			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Production of biofilm Biofilm producers	20	90.9	2	9.1	22	100.0	18	81.8	4	18.2	22	100.0
Non biofilm producers	6	21.4	22	78.6	28	100.0	6	21.4	22	78.6	28	100.0
X2 (p)	**23.828* (<0.001*)**						**18.001*(<0.001*)**					
Production of ESβL ESβL producers	16	59.3	11	40.7	27	100.0	14	51.9	13	48.1	27	100.0
Non ESβL producers	10	43.5	13	56.5	23	100.0	10	43.5	13	56.5	23	100.0
X2 (p)	**1.239 (0.266)**						**0.349 (0.555)**					

Table 5. Concordance of Minimal inhibitory and Minimal bactericidal concentrations of Silver Nano particles between biofilm producers and non -biofilm producers

Concentration of silver nano particles	Total (n=50)		Biofilm producers (n=22)		Non-biofilm producers (n=28)		$\chi2$	p value
	No.	%	No.	%	No.	%		
MIC=MBC	34	68.0	10	45.5	24	85.7	9.177[*]	0.002[*]
6.25µg/ml	18	36.0	0	0.0	18	64.3	22.098[*]	<0.001[*]
12.5 µg/ml	10	20.0	4	18.2	6	21.4	0.081	p=1.000
25 µg/ml	6	12.0	6	27.3	0	0.0	8.678[*]	p=0.005[*]
MIC<MBC	16	32.0	12	54.5	4	14.3	9.177[*]	0.002[*]
MIC (6.25 µg/ml) < MBC (12.5 µg/ml)	10	20.0	7	31.8	3	10.7	3.429	p=0.084
MIC (12.5 µg/ml) < MBC (25 µg/ml)	4	8.0	3	13.6	1	3.6	1.696	p=0.308
MIC (25 µg/ml) < MBC (50 µg/ml)	2	4.0	2	9.1	0	0.0	2.652	p=0.189

Figure (1a): Agarose gel electrophoresis for the PCR amplified products of *Pseudomonas aeruginosa* pslA gene.
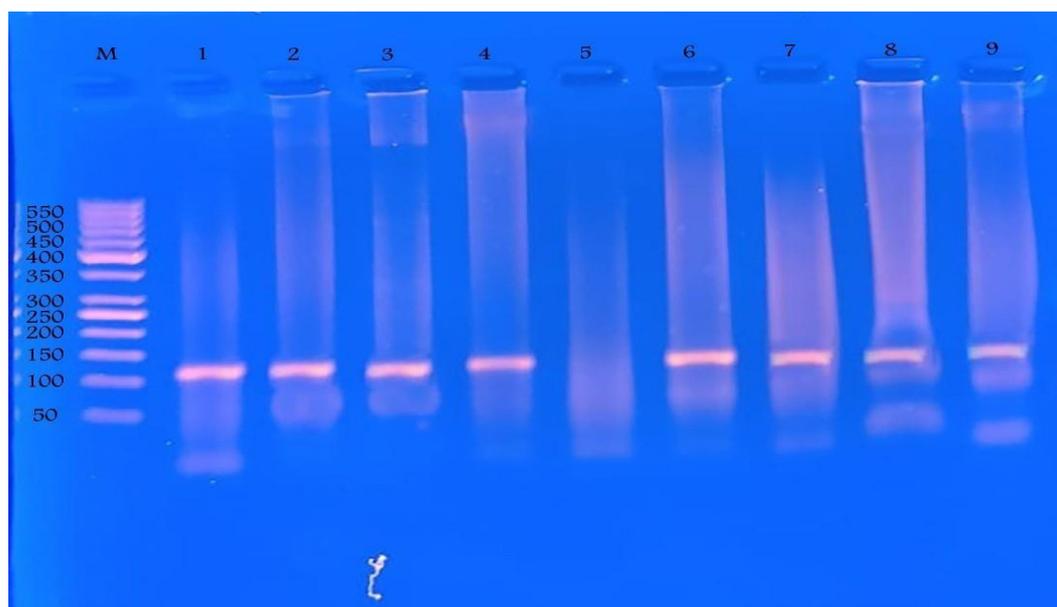
Lane M: DNA molecular size marker (50-550 bp).
Lanes 1, 2, 3, 4, 6, 7,8and 9 were positive for pslA gene (119 bp).
Lanes 5 was negative for pslA gene (119 bp).

Figure (1b): Agarose gel electrophoresis for the PCR amplified products of lasR gene.
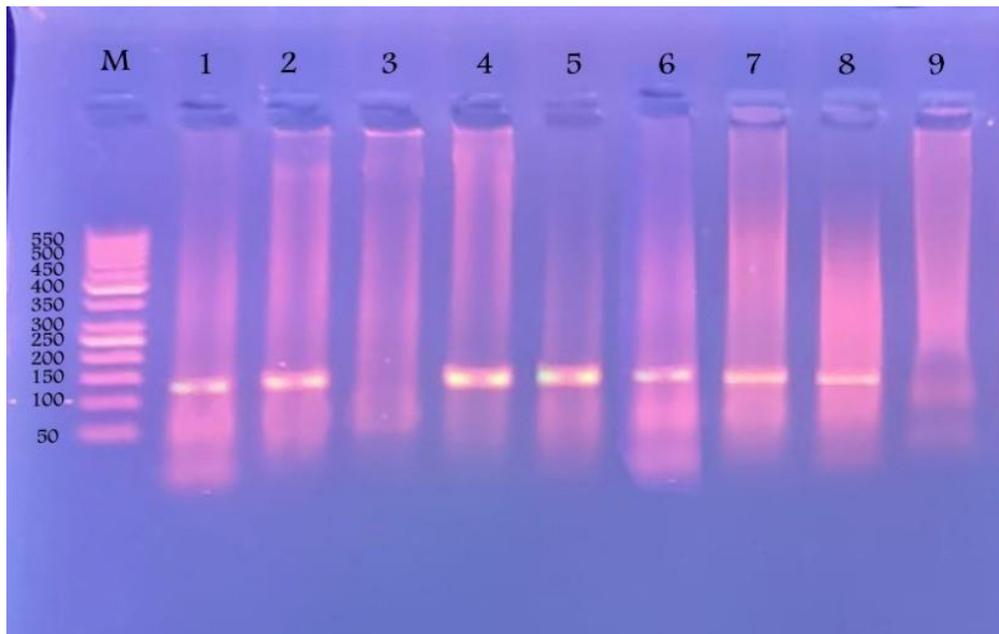
Lane M: DNA molecular size marker (50-550bp).
Lanes 1,2, 4,5, 6,7 and 8 were positive for lasR gene (130 bp).
Lanes 3 and 9were negative for lasR gene (130 bp).

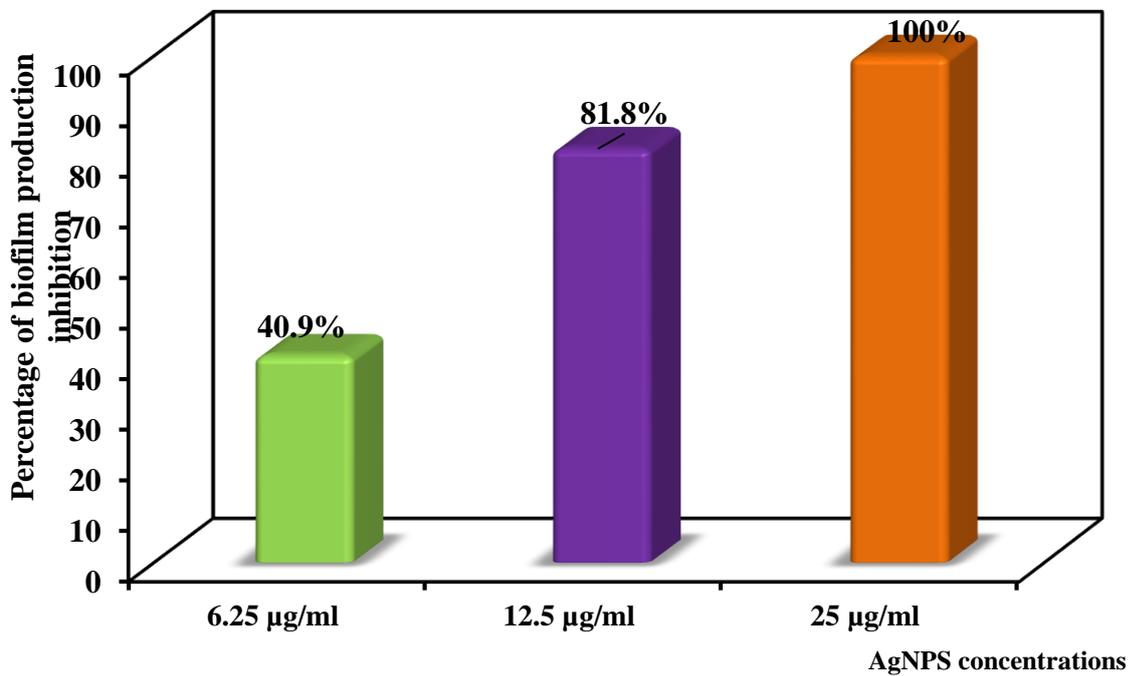**Figure (2a):** Effect of different concentrations of AgNPS on biofilm forming *P. aeruginosa*.

Figure (2b) Anti-biofilm concentration of AgNPs on biofilm forming isolates on MCRA that show there is a biofilm at 6.25 and 12.5 µg/ml and absence of biofilm and growth of organism at concentration of 25 and 50 µg/ml.

Discussion

The Centers for Disease Control and Prevention (CDC) have considered multidrug-resistant *P. aeruginosa* as a serious threat for the past ten years [19].

P. aeruginosa were isolated from 16.7% of clinical samples. This result was similar to Mahmoud et al., [20] study performed in Egypt that found 19%; (54/283) of all specimens were *P. aeruginosa.* On the other hand, a higher percentage (33.3%) was reported by Abd El-Baky et al., [21] in Egypt In contrast, a very low percentage (6.67%) was found by Khan et al., [22] in Pakistan. These differences may be due to differences in adherence to infection control measures and variable resistance to antibiotics [22].

P. aeruginosa was mostly isolated from burn wounds (60%). This was consistent with Roshani et al., [23] findings, which demonstrated that burn samples accounted for a significant source proportion of the isolates (58%). This high prevalence may be due to that *P. aeruginosa* can colonize the skin and mucous membranes of burn patients and form biofilms that protect the bacteria from host defenses and antibiotics [24].

About 62% of the isolates in this study were ceftazidime resistant. A similar result (61.4%)

was obtained by Kos et al., [25] in China, resistance to aminoglycosides in our study was similar to the study conducted by Asghar and Ahmed, [26] in KSA which found resistance rate ranged from 46% to 52%. Resistance to Quinolones (ciprofloxacin, levofloxacin, and ofloxacin) varied from (78% to 92%) in our study. Similarly, a study performed in Iran by Arabameri et al. [27] revealed that 78% of isolates were resistant to levofloxacin and 80% were resistant to the ciprofloxacin. For meropenem and imipenem respectively about 68% and 60% of *P. aeruginosa* isolates showed sensitivity. A comparable result was obtained by Pokharel et al., (28) in Nepal in which 65% of isolates were susceptible to both imipenem and meropenem.

In the current study, screening of ESβL production showed that 62% of isolated *P. aeruginosa* were ESβL producers, whereas the combined disk test detected 54%. Woodford et al. [29] in the United Kingdom reported near findings, where 47% of *P. aeruginosa* isolates were ESβL-positive.

In this study, 22/50 (44%) of *P. aeruginosa* isolates were biofilm producers of which 20/22 (90.9%) formed strong biofilms and 2/22 (9.1%) formed weak biofilms. Heydari and Eftekhar, [30] in Iran published that 43.5% of isolates were biofilm producers of which 66.7% produced strong biofilms

and 33.3% formed weak biofilms. A higher rate of biofilm production among *P. areuginosa* isolates was reported by El-Khashaab et al., [31] in Egypt (91.4%). In contrast, lower rates of biofilm production among *P. aeruginosa* isolates were reported by Abootaleb et al., [4] in Iran (24%). The variance could be explained by the various types and numbers of samples used in each study and the isolates' capacity to form a biofilm [32].

In the present study. pslA gene was detected in 20/22 (90.9%) of biofilm-producing *P. aeruginosa* isolates. Nader et al. [33] observed a higher rate in Iraq, where 31/31 (100%) of biofilm-forming isolates had the pslA gene. As for lasR gene, it was found in 18/22 (81.8%) of biofilm-producing *P. aeruginosa* isolates. Similarly, Abdelraheem et al., [32] in Egypt documented that lasR was detected in 20/27 (74.1%) of biofilm-forming isolates. Higher result (84%) was found by Elnegery et al., [34], while lower rates (40%) was noted by Aboushleib et al., [35] in Egypt.

When evaluating the anti-bacterial activity of AgNPs, the MIC of AgNPs ranged from 6.25 to 25 µg/ml with a better antibacterial activity as compared to the earlier work of Singh et al. [36], who observed that the MIC of AgNPs ranged from 6.25 to 200 µg/ml, On contrast, Liao et al., [37] in china reported lower MIC range (2.812-5.624 µg/mL) of AgNPs.

The major application of AgNPs is as a biofilm inhibiting agent against *P. aeruginosa* isolates. AgNPs were most effective in biofilm prevention among *P. aeruginosa* at a concentration of 25 µg/ml, with a 100% inhibition rate. However, Kalishwaralal et al., [38] revealed that the maximal inhibition of biofilm was achieved at concentration 100 µg/mL. According to Singh et al., [39] in Denmark, 50 µg/mL of AgNPs had accepted anti-biofilm effect against *P. aeruginosa*. Moreover, Palanisamy et al. [40] in Malaysia found that the maximal activity of AgNPs against biofilm development occurred at a concentration of 25 µg/ml with an inhibition rate of 67%.

Conclusion and Recommendations

Recently frequent serious nosocomial infections could result from multi-drug resistant *P. aeruginosa* isolates which positively correlated with biofilm-forming capacity of the isolate. There is a high prevalence of the pslA and lasR genes among biofilm forming isolates. The synthesized AgNPs showed both antibacterial and anti-biofilm activity.

More studies must be performed to show the safety and antimicrobial activity of AgNPs.

Limitation

The inability to cultivate the samples anaerobically due to financial constraints was one of the study's limitations.

Conflict of interest. No hidden conflicts of interest for the writers to mention.

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