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Antibacterial Potential of a Newly Synthesized Zinc Peroxide Nanoparticles (ZnO_2-NPs) to Combat Biofilm-Producing Multi-Drug Resistant *Pseudomonas aeruginosa*

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THE SPREAD of resistant bacteria and the development of bacterial biofilm are two major challenges in the application of biomaterials. The overuse of antibiotics has become a common cause of the emergence of multidrug-resistant (MDR) bacteria. Besides, biofilm infections are notoriously difficult to treat, as the biofilm matrix provides physical protection from antibiotic treatment. Therefore, a variety of new antimicrobial drugs has attracted wide attention in treating infectious diseases developing from MDR bacteria and bacterial biofilms. These drugs are related to an important group based on the use of nanoparticle-based materials. Metal oxide nanoparticles including zinc peroxide nanoparticles (ZnO_2-NPs) exhibit remarkable antimicrobial activities against MDR bacteria and hence are one of the most propitious alternative antimicrobial agents. Herein, the antibacterial activity of the synthesized ZnO_2-NPs was investigated against 7 clinical MDR *Pseudomonas aeruginosa* strains using disc diffusion assays on Muller-Hinton agar. These strains were also multi-virulence producers with special reference to hemolysin, pyocyanin, gelatinase, protease, lipase and biofilm production. Clearly, a significant bactericidal activity of ZnO_2-NPs against tested strains was exhibited, with a maximum zone of inhibition of 19.81±1.5mm against *P. aeruginosa* strain 22 (PA-22) at a concentration of 300µg/ml. In addition, ZnO_2-NPs exhibited a significant anti-biofilm activity by inhibiting bacterial biofilm formation as revealed spectrophotometrically. This study established the possibility of developing antimicrobial ZnO_2-NPs to combat developing drug resistance and biofilm-related infections.

Keywords: *Pseudomonas aeruginosa*, Zinc peroxide nanoparticles, Multi-drug resistance, Biofilm.

Introduction

Multidrug-resistant (MDR) bacteria remain the greatest challenge in public health care. The numbers of infections produced by such resistant strains are increasing globally. This acquired resistance of pathogens presents a key challenge for many antimicrobial drugs (Ali et al., 2019). Such multidrug-resistant (MDR) microbes make the treatment more difficult and expensive with more side effects. Many diseases showed difficulties in their treatment after the advent of MDR bacteria due to the use of higher dose and potent antibiotics (El-Zawawy & Ali, 2016a). The situation continues to be more alarming due to meager efforts put to develop new drugs (El-Badry & Ali, 2015; Ali et al., 2016; Al-Tohamy et al., 2018). Since 2000 to 2013, almost 22 new antibiotics had been developed to overcome MDR phenomenon and yet antibiotic resistance still persists. The major difficulties are: The isolation of novel antibiotics, prolonged development time, immense clinical trials cost, and most importantly the emergence of resistance against newly developed compounds

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(Ali et al., 2014). World Health Organization (WHO, 2017) has placed three pathogens in the newly revised list of critical priority pathogens. It includes carbapenem-resistant pathogens, i.e., *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and all other Enterobacteriaceae that display resistance against carbapenems. WHO has urged the need to develop new antibiotics against these three pathogens on a priority basis.

Pseudomonas aeruginosa, a Gram-negative bacterium, is one of the bacteria found to be resistant to multiple antibiotics (El-Shouny et al., 2016). It rarely causes problems in healthy individuals, however, in immunocompromised patients, it is one of the most important opportunistic pathogens that contribute to the high rate of morbidity and mortality (Goossens, 2003). Recent advances in nanotechnology offer new prospects to develop novel formulations based on distinct types of nanoparticles (NPs) with different sizes and shapes and flexible antimicrobial properties (Ali et al., 2017). NPs may offer a promising solution as they can not only combat bacteria themselves but can also act as carriers for antibiotics and natural antimicrobial compounds (Wang et al., 2017). While various materials have been explored from liposomal to polymer-based nano-drug carriers, metallic vectors, such as gold NPs, are attractive as core materials due to their essentially inert and nontoxic nature (Ali et al., 2017). Metal oxides NPs are the most explored and studied families of NPs and are known to be effectively inhibit the growth of a wide range of sensitive and resistant bacteria, emerging as hopeful candidates to challenge antimicrobial resistance (Kadiyala et al., 2018). Improvement of the pharmacokinetic profile and therapeutic index of encapsulated drugs can be dramatically decrease the dose required to achieve clinical effects (Gao et al., 2018). This in turn, can reduce the toxicity and the adverse side effects associated with high systemic drug concentrations and frequent dosing (Liu et al., 2009).

Nano-sized ZnO exhibits varying morphologies and shows significant antibacterial activity over a wide spectrum of bacterial species explored by a large body of researchers. ZnO is currently being investigated as an antibacterial agent in both microscale and nanoscale formulations. ZnO exhibits significant antimicrobial activities when the particle size is reduced to the nanometer range, i.e., in nan-sized scale. Then nano-sized ZnO can interact with bacterial surface and/or with the bacterial core where it inters inside the cell, and subsequently exhibits distinct bactericidal mechanisms (Seil & Webster, 2012). The interactions between these unique materials and bacteria are mostly toxic, which have been exploited for antimicrobial applications such as in food industry.

Interestingly, ZnO_2-NPs are reported by several studies as non-toxic to human cells, this aspect necessitated their usage as antibacterial agents, noxious to microorganisms, and hold good biocompatibility to human cells. The various antibacterial mechanisms of nanomaterials are mostly attributed to their high specific surface area-to-volume ratios (Ali et al., 2017; Seil et al., 2009) and their distinctive physico-chemical properties. However, the precise mechanisms are yet under debate, although several proposed ones are suggested and adopted. Investigations on antibacterial nanomaterials, mostly ZnO-NPs, would enhance the research area of nanomaterials and the mechanisms and phenomenon behind nanostructured materials. This study aims todesign and characterize ZnO_2-NPsas a novel antimicrobial approach combating biofilm-producing multi-drug resistant *Pseudomonas aeruginosa* infections.

Materials and Methods

Isolation, identification, selection and confirmation of MDR bacterial isolates

A total of 130 clinical specimens were isolated from ear, urine, sputum, wounds and burns of patients attending Tanta General Hospital and one reference strain of *Pseudomonas aeruginosa* ATCC 25375 (kindly provided by Dr. Sameh Samir Ali, Asssociate Professor of Microbiology, Faculty of Science, Tanta University, Egypt).

The clinicians followed the guidelines and the standard protocols that are compatible with the requirements of the Declaration of Helsinki. Specimens were immediately placed in nutrient broth (NB) transport media and then transferred to the laboratory of Bacteriology at Faculty of Science, Tanta University. Each specimen was cultured on nutrient agar and blood agar plates. Colonies that eventually grew in these media were sub-cultured on MacConkey's agar. Non lactose fermenting colonies were further sub-cultured on

cetrimide agar plates supplemented with 15µg/ml nalidixic acid for preliminary selection of *P. aeruginosa* isolates (El-Shouny et al., 2015; Khalil et al., 2015) and were examined for different morphological (size, shape and Gram reaction) and biochemical characterization (oxidase test, catalase test, oxidation-fermentation test, nitrate reduction test, methyl red test, arginine hydrolysis and growth at 42°C) for identification to the species level as described by Cowan and Steel's Manual for the Identification of Bacteria (Barrow & Feltham, 1993) and Bergey's Manual for Systematic Bacteriology (Krieg & Holt, 2001).

The Multi-drug resistance of clinical isolates was confirmed with VITEK 2® Compact automated system (Bio Mérieux, Marcy l'Etoile, France) in Mabaret EL Asafra Hospital (Alexandria, Egypt) using GN Test Kit VTK2/ GP Test Kit VTK2. All bacterial isolates were maintained in brain heart infusion (BHI) broth containing 15% glycerol at -75°C through the study period.

Phenotypic expression of virulence factors

A total of 35 *P. aeruginosa* isolates were tested for their ability ofhaemolysin production using blood agar media and in protease production (casein hydrolysis) using skimmed milk agar as described by Madigan et al. (1999). Gelatin production was tested by inoculating tubes containing nutrient gelatin medium via a straight-line inside of tubes containing medium according to MacFaddin (1985). For Tween 80 hydrolysis, Tween 80 agar plates were inoculated by each tested isolate and were checked each day. Opaque zones surrounding the inocula were indicative of tween 80 hydrolysis (Pavlov et al., 2004). In order to identify general pigment production, the isolates were streaked on Fluka Pseudomonas isolation agar F (PIA F, Fluka) to detect fluorescein production and Fluka Pseudomonas isolation agar P (PIA P, Fluka) to detect pyocyanin production. All plates were incubated for 48hr at 37ºC and the produced colors by the isolates were recorded (Brooks et al., 2007).

Anti-biofilm assay

Biofilm production by *P. aeruginosa* was estimated qualitatively for all the isolates by the tube method as described previously by Christensen et al. (1985). Two or three colonies were inoculated into 5ml of BHI broth in glass tubes. Cultures were incubated at 37ºC for 18-20hr and the culture contents were aspirated. Tubes were stained with safranin and presence of a visible stained film lined the wall and bottom of the tube was considered to be positive for slime production. Ring formation at the liquid interface was not considered as indicative of biofilm formation. To compare observer variation with the tube method, the observations were compared with each other and with spectrophotometric measurements at 570nm. Different concentrations (100, 200, 300, 400 and 500µg/ml) of ZnO_2-NPs were prepared by suspending the nanoparticles in double-distilled water and the technique was carried out according to the method described by Sangani et al. (2015) to detect the efficacy of ZnO_2-NPs on formed biofilm

Antimicrobial susceptibility test for selecting MDR strains

Susceptibilities of the selected isolates were tested against 13 antimicrobials of different classes using the Kirby–Bauer method on Mueller Hinton agar with commercially available antimicrobial disks (Oxoid, UK), according to Clinical Laboratory Standards Institute (CLSI, 2010). *P. aeruginosa*, multidrug resistance (MDRPA) defined as resistance to at least three of sixdrugs, including amikacin, gentamycin, ciprofloxacin, piperacillin,ceftazidime and imipenem (El-Shouny et al., 2018). According to this definition the bacterial isolates (MDR) were selected.

Synthesis of ZnO_2-NPs

ZnO_2-NPs were synthesized according to the methods described by Ali et al. (2017) using the following analytical grade chemicals without further purification: Zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$], ammoniumhydroxide ($NH_4OH$), hydrogen peroxide ($H_2O_2$; sol 40%), glycerol, ethanol and acetone (Adwic, El-nasr Chemical Co., Cairo, Egypt).

In a typical procedure, NH_4OH (10ml) was mixed with 20ml of 0.1mol of Zn $(CH_3COO)_2 \cdot 2H_2O$ under magnetic stirring. A volume of 70ml of acetone and 3g glycerol were rapidly added to the suspension. Then 40ml of H_2O_2 was added to the suspension with stirring for 30min at room temperature. The suspended precipitate was centrifuged and washed 3 times with distilled water.

Antimicrobial activity of ZnO_2-NPs

In vitro antimicrobial activity of synthesized ZnO_2-NPs was carried out by disc diffusion assays on Muller-Hinton agar. Different concentrations (300, 200, 100 and 50µg/ml) of ZnO_2-NPs impregnated filter paper discs (6mm) were tested against 7 clinical MDR *P. aeruginosa* strains.

Minimum inhibitory concentration (MIC) was performed by a serial dilution technique (Lalitha et al., 2010) using 96-well microtiter plates. Various concentrations (200, 100, 50, 25, 20, 15 and 10µg/ml) of ZnO_2-NPs were prepared, and 2µL of the prepared inoculum suspension was added to every well. The plates were incubated at the optimal conditions. MIC was the lowest concentration of ZnO_2-NPs at which no visible growth was observed. For determining minimum bactericidal concentration (MBC); a volume of the aliquots (10µL) from the wells, which was used in MIC assays and showed no turbidity, was sub-cultured on the surface of the nutrient agar. MBC was defined as the lowest concentration of the ZnO_2-NPs at which there was no colony formation of *P. aeruginosa* after 18hr incubation period.

Statistical methods

PC-ORD for windows (ver.5) was used for two ways hierarchical cluster analysis using Sorensen methods for distance and beta (-0.025) for group linkage. The data were collected, tabulated and statistically analyzed using Minitab 17.1.0.0 for windows (Minitab Inc., 2013, Pennsylvania, USA). All tests were two-sided. A P value< 0.05 was considered significant. Data normality was checked for using Shapiro-Wilk test. One and two way (ANOVA) tests used to compare between more than two groups with multiple comparisons using Tukey methods.

Results and Discussion

Isolation, identification, selection and confirmation of MDR clinical isolates

The isolates were identified according to the morphological characters of colonies grown on cetrimide agar medium followed by biochemical characterization tests. Morphological characterization exhibited circular to oval colonies, large with entire or undulate edges, flat or raised convex elevated appearance, smooth and with time they tended to spread on the surface of the agar. All isolates were identified as being Gram-negative rods. All isolates were subjected to biochemical characterization. The obtained data indicated that all the isolates were able to grow at 42°C but not at 4°C and exhibited positive results in oxidase, catalase and arginine hydrolysis. Also, the isolates were all oxidative organisms when grown on Hugh and Liefson′s medium and were able to reduce nitrate to nitrite, however, they showed negative results for methyl red test. Figure 1 shows the relative abundance of specimens and *P. aeruginosa* isolates. The specimens from burns were the most abundant (39%), followed by wound and sputum; 19 and 15%, respectively. The prevalence of *P. aeruginosa* was arranged regarding its abundance in clinical samples, as follows: In burn (13%), followed by sputum and wound by 5% and finally in urine by 4%. The multi-drug resistance of clinical isolates was confirmed with VITEK 2® Compact automated system. The selected 7 representative MDRPA isolates were also confirmed phenotypically as *P. aeruginosa* with 98-99% probability percentage.

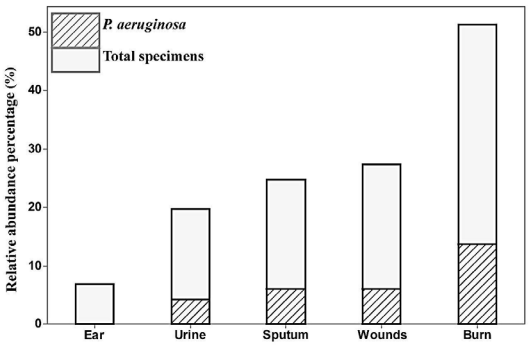

Fig. 1. Prevalence of *P. aeruginosa* isolates in different clinical sources.

Phenotypic expression of virulence factors

The pathogenesis of *P. aeruginosa* is due to several virulence factors. This organism produces several extracellular products that after colonization can cause extensive tissue damage, bloodstream invasion and dissemination. Proteases are assumed to play a major role during acute *P. aeruginosa* infection (El-Zawawy & Ali, 2016b). The *in vitro* phenotypic expression performed in this study revealed production of all the six virulence factors by the majority of isolates.

The cluster dendrogram (Fig. 2) showed that; 23 of 35 isolates had more than 50% of all six examined virulence factors, from that 23 isolates, only two isolates were positive to all investigated virulence factors and the reaming 21 isolates had virulence activity above 50% and less than

100%. The most prominent virulence factors werea hemolytic activity (91.43%), followed by biofilm formation (71.43%), pyocyanin production (65.71%), then protease and lipase production (57.14 and 54.29%), respectively, finally gelatinase production occured in only 40% of tested isolates.

It is well established that biofilm-forming bacteriaare more resistant to antimicrobial agents than their planktonic counterparts. The biofilm is composed of alginate and confers a mucoid consistency to *P. aeruginosa* isolates, acting as a protecting niche for the bacterium against the recognition of the immune system and the action of antibiotics. All these factors increase the possibility of chronic infections. In the same concern, Minhas et al. (2015) reported that 90% of all isolates showed gelatinase activity and 60% were hemolysin evidenced, by hemolysis on blood agar, 70% were found to express protease activity, while 66.67% and 83.33% were biofilm producers. Jácome et al. (2012) indicated that 93.4%, 72.1% and 34.4% of *P. aeruginosa* strains having gelatinase, hemolysin and biofilm production activities, respectively. P. *aeruginosa* also produces some proteases (LasB elastase, LasA elastase and alkaline protease) which are able to destroy the protein elastin. This protein forms a large constituent of human lung tissue that is responsible for lung expansion and contraction. However, Deptula & Gospodarek (2010) observed, even with smaller percentages, 9.3% multidrug susceptible and 8% multidrug-resistant isolates as biofilm producers which are lesser as compared to our study.

Susceptibility of P. aeruginosa isolates to different anti-microbial agents

The antimicrobial susceptibility profiles observed in this study revealed that 94.29% isolates of *P. aeruginosa* were sensitive to oxacillin and lesser proportions of the isolates were susceptible to imipenem (22.86%) as shown in Table 1. In this context, Minhas et al. (2015) revealed that 90% isolates of *P. aeruginosa* were sensitive to piperacillin/tazobactam, 75% to piperacillin and 68.89% to imipenem. Amutha et al. (2009) reported the highest resistance of *P. aeruginosa* strains to ampicillin (85%) and imipenem (5%). Gad et al. (2008) reported that *P. aeruginosa* skin infection isolates were 100% resistant to ampicillin and amoxicillin. As shown in Pie chart (Fig. 3), only 23 % of *P. aeruginosa* isolates showed sensitivity response to drugs, and about 77% exhibited drug resistance pattern toward different antimicrobial agents; 57.1 and 20% were MDR and pan-drug resistant, respectively. Pan-drug resistant (PDR) was resistant to all antibiotic agents (Magiorakos et al., 2012). In the same concern, Minhas et al. (2015) reported that 27.22% *P. aeruginosa* isolates were MDR, 55% were extensively drug-resistant (XDR) and 1.11% were PDR isolates. Dash et al. (2014) reported a high percentage (84.7%) of MDR *P. aeruginosa* strains and a moderate percentage of 35.7% XDR *P. aeruginosa* strains. Chauhan & Sharma (2013) recorded 69.5% *P. aeruginosa* isolates as multidrug-resistant and did not observed any PDR isolates

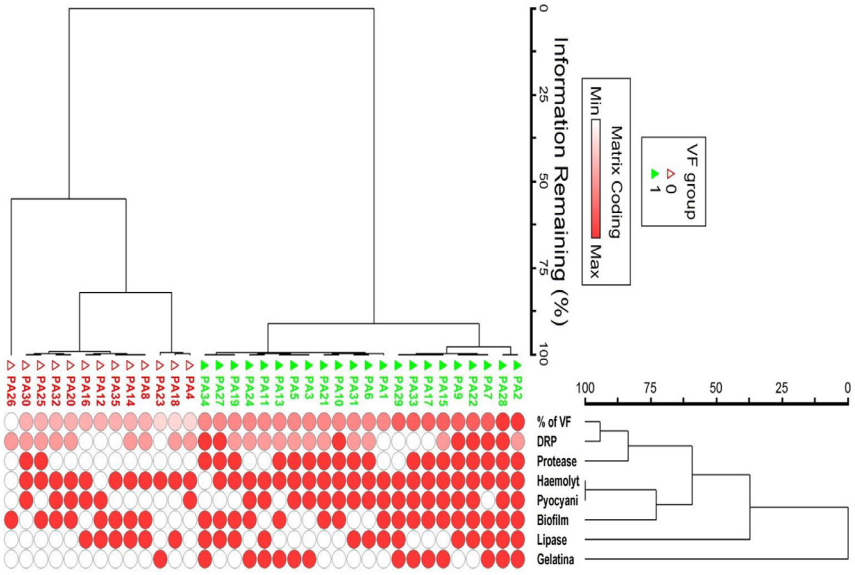

Fig. 2. Virulence factors in *P. aeruginosa* isolate.

TABLE1. Incidence of resistance of *P. aeruginosa* isolates to different anti-microbial agents.

Anti-microbial drug	No (%) of resistant isolates
Imipenem	8 (22.86)
Amikacin	11 (31.43)
Ciprofloxacin	15 (42.86)
Chloramphenicol	17 (48.57)
Ceftriaxone	18 (51.43)
Kanamycin	26 (74.29)
Co-trimoxazole	27 (77.14)
Ceftazidime	28 (80.00)
ColistinSulphate	28 (80.00)
Streptomycin	30 (85.71)
Aztreonam	31 (88.57)
Tetracycline	31 (88.57)
Oxacillin	33 (94.29)

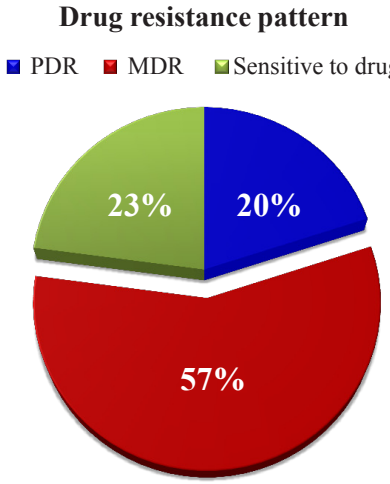

Fig. 3. Drug resistance pattern.

Antimicrobial activity of ZnO_2-NPs against MDR strains

Several reports investigated the effect of ZnO-NPs as antibacterial due to their unique properties (Siddiqi et al., 2018). However, the present study investigates and evaluates the effect of ZnO_2-NPs against MDR pathogens isolated from ear, urine, sputum, wounds and burns. The data obtained herein are listed in Table 2. The data revealed the anti-microbial activities of synthesized ZnO_2-NPs against MDR *P. aeruginosa* and showed that PA-22, 27 and 9 were highly susceptible and had the highest and significant inhibition zone than other tested strains, regardless the concentration of ZnO_2-NPs. However, one strain (PA-07) showed no sensitivity to ZnO_2-NPs. In a conclusion, the ZnO_2-NPs showed significant inhibitory activity with notable differences in the susceptibility to ZnO_2-NPs in a dose-dependent manner. The mean zones of inhibition ranged from 2.25±0.5 to 19.81±1.5mm. MIC and MBC are shown in Table 3. The recorded MIC values ranged between 10 and 50µg/ml, meanwhile, MBC values ranged between 20 and 100µg/ml. The increase in ZnO_2-NPs concentration (50, 100, 200 and 300µg/ml) was correlated with increasing antimicrobial activities. This may be due to the increased H_2O_2 concentration from the surface of ZnO_2. By this way, the generated H_2O_2 can penetrate the cell membranes of *P. aeruginosa* causing a lethal effect. From the obtained results as shown in Fig. 4, the optical density (OD) of biofilm at 570nm was significantly decreased with increasing the concentration of ZnO_2-NPs. Wang et al. (2017) explained that metal oxides slowly release metal ions that are uptaken by the cell, reaching the intracellular compartment where they can interact with functional groups of proteins and nucleic acids, such as amino (–NH), mercapto (–SH) and carboxyl (– COOH) groups. This interaction alters the cell structure, hampers enzymatic activity and interferes with the normal physiological processes in the bacterial cells. In the same concern, Sangeetha & Kumaraguru (2013) explained the mechanism of anti-microbial activities of metal oxide NPs. Small particle size of metal oxide NPs is associated with a larger band gap; consequently, these unfavorable conditions can prevent the recombination of excitons. Therefore, more available excitons will result in the formation of a higher concentration of reactive oxygen species and consequently, enhance the antimicrobial activities of metal oxide NPs.

TABLE 2. Antibacterial activity of ZnO_2-NPs against MDR *P. aeruginosa* strains.

Strain	Inhibition zone (mm) of strain by ZnO_2-NPs (µg/ml)	
	Mean	StDev
PA-7	0	0
PA-9	11.9	1.0
PA-10	9.05	0.5
PA-22	16.55	0.2
PA-27	15.52	0.2
PA-28	7.75	0.5
PA-34	5.08	0.6
P-value	<0.001	

Two way ANOVA test with multiple comparison with Tukey methods, P considered significant if P< 0.05.

TABLE 3. Antimicrobial activity of ZnO_2-NPs against MDR *Pseudomonas aeruginosa*.

Strain code	Diameter of inhibition zone (mm)				MIC (µg/ml)	MBC (µg/ml)
	Concentrations of ZnO_2-NP (µg/ml)					
	50	100	200	300		
PA-7	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	ND	ND
PA-9	4.25±0.50[b]	6.35±0.90[b]	8.26±0.60[b]	10.75±0.85[b]	50	100
PA-10	3.50±0.50[b]	4.25±0.85[b]	6.25±0.80[b]	8.22±1.10[b]	25	50
PA-22	8.34±1.00[c]	12.20±1.0[c]	15.27±0.75[c]	19.81±1.50[c]	10	20
PA-27	6.20±0.80[b]	8.25±1.2[b]	10.52±1.50[b]	15.50±0.95[c]	15	30
PA-28	2.25±0.50[b]	4.22±0.00[b]	6.15±0.50[bd]	11.25±1.00[b]	10	20
PA-34	2.85±0.50[b]	3.22±0.50[b]	4.25±0.50[d]	8.10±0.50[b]	50	100

- Values are the mean of three replicates±SD.
- Means with the same letters in the same column show the insignificant difference (P considered significant if P≤0.05).

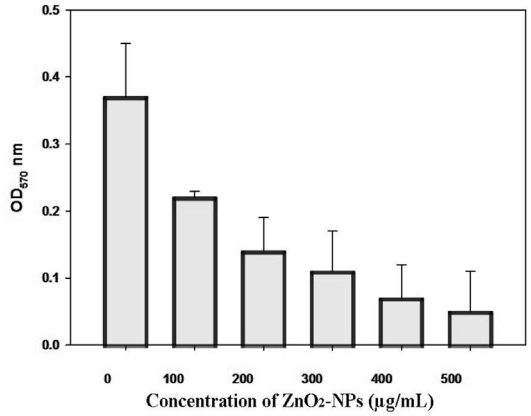

Fig. 4. Antibiofilm activity by different concentration ZnO_2-NPs against PA-22 strain.

Conclusion

Nanobiotechnology has emerged as an efficient technology for the development of antimicrobial nanoparticles through an eco-friendly approach. In this study, the antibacterial activity of ZnO_2-NPs was investigated against MDR *P. aeruginosa* strains through disc diffusion assays on Muller Hinton Agar. Antimicrobial activities of ZnO_2-NPs increased with the increase of concentrations. Moreover, our results reported the reduced pathogenicity and biofilm-producing strains. This study thus established the possibility of developing antimicrobial ZnO_2-NPs to combat developing drug resistance and biofilm-related infections. More *in vivo* investigations are needed on the experimental animal models with skin burns to confirm the efficacy of ZnO_2-NPs as a novel antimicrobial drug in wound healing, especially after the success of ZnO_2-NPs in the *in vitro* against MDR *P. aeruginosa*.

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قدرة جسيمات بيروكسيد الزنك (ZnO_2-NPs) المصنعة حديثا في مكافحة بكتيريا سيدوموناس اورجينوزا المقاومة للعديد من المضادات الحيوية والمنتجة للأغشية البكتيرية

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يمثل انتشار البكتيريا المقاومة وتطور الأغشية البكتيرية تحديين رئيسيين في التطبيق الحيوي للمواد. أصبح الإفراط في استخدام المضادات الحيوية سببًا شائعًا لظهور بكتيريا مقاومة للعديد من المضادات الحيوية (MDR). إلى جانب ذلك، فأنه يصعب علاج العدوى الناتجة من الأغشية البكتيرية لأنها توفر حماية طبيعيه ضد المضادات الحيوية. لذلك، كان هناك اهتمام كبير لمجموعة جديدة من مضادات الميكروبات، والتي تستخدم في علاج الأمراض الناتجة من البكتريا المقاومة (MDR) والأغشية البكتيرية. ترتبط هذه العقاقير بمجموعة مهمة تعتمد في الأساس على استخدام الجسيمات النانوية. الجسيمات النانونية الأوكسيدية مثل بيروكسيد الزنك (ZnO_2-NPs) لها تأثير مضاد للميكروبات وبالتالي تعتبر أحد أهم العوامل البديلة ضد البكتريا المقاومة (MDR) والأغشية البكتيرية. تناولت هذه الدراسة نشاط جسيمات بيروكسيد الزنك (ZnO_2-NPs) النانوية ضد سبعه عزلات سريرية من البكتريا المقاومة سيدوموناس اورجينوزا (*Pseudomonas aeruginosa* MDR) ، باستخدام طريقة القرص المنتشر (disc diffusion assays) على أطباق الآجار (Muller-Hinton agar). كانت هذه السلالات البكتيرية أيضًا مسببة للعدوى المتعددة في إشارة خاصة إلى الهيموليسين والبيوسيانين والجيلاتيناز والبروتياز والليباز وإنتاج الأغشية البكتيرية. أظهرت النتائج نشاط ملحوظ لجسيمات بيروكسيد الزنك (ZnO_2-NPs) ضد السلالات البكتيرية المختبرة، مع أقصى منطقة تثبيط تبلغ 19.81 ± 1.5 مم ضد السلاله رقم 22 (PA-22) بتركيز 300 ميكروجرام/مل. بالإضافة إلى ذلك، أظهرت جسيمات بيروكسيد الزنك (ZnO_2-NPs) نشاطا كبيرا ضد تكوين الأغشية البكتيرية. توضح هذه الدراسة إمكانية تطوير جسيمات بيروكسيد الزنك (ZnO_2-NPs) لمكافحة العدوى الناتجة من البكتريا المقاومة للمضادات الحيوية (MDR) والأغشية البكتيرية.