Antimicrobial and Biofilm Inhibitory Activity of Nanoparticles Against Clinical Isolates from Urinary Tract Infection

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Abstract

At these days nanoparticles (NPs) are used as antibacterial due to its have chemical-physical addition to biological effect. A big group of microbial cells adhering to a surface are called biofilm. Exposure to nano particles such as (Ag, Al_2O_3, Ni) may prevent colonization of new bacteria onto the biofilm. In the present research we have analyze whether the biofilm formation of some isolates of pathogenic bacteria could be influenced by NPs. Also we examined the susceptibility of the isolates to some antibiotics in combination with Ag, Al_2O_3 and Ni nanoparticles. (50) Strains of isolated *K. pneumonia* have been examine from patients with urinary tract infection were collected from Education of Baghdad hospitals in Iraq. Serial dilution of Tube method to determination of MIC and MBEC for Nanoparticles against isolates was done. Biofilm formation was evaluated by micro titer plates. Additionally. disc diffusion method was used to assay the various antibiotics and combinations for bactericidal activity against the isolates. The results showed that about 97% of the strains were capable to form of structure biofilms. More than 82% about of strains showed resistance to commonly used antibiotics. Antibiotics and antimicrobials from mixed nanoparticles were higher than compared to nanoparticles alone. Therefore, a synergistic effect was observed between silver - Nickel nanoparticles and silver - aluminum oxide nanoparticles in to inhibiting the growth of K. pneumonia strains. Furthermore, cytotoxicity activity of Ag NPs against Neuro-2a cells was higher than Al_2O_3 NPs. The results suggest that nanoparticles can be used as antimicrobial agents for therapeutic to treat urinary tract infections caused by *K. pneumoniae*,

Keywords: *Antimicrobial activity, anti-biofilm, K. pneumoniae, Urinary Tract Infection, Nanoparticles.*

Introduction

Urinary tract infections are one of the most common infectious diseases, mainly caused by gram negative bacteria it is estimated that klebsella species account for about 8% from all infectious diseases in the USA and Europe (1). Klebsella infection is mainly caused by *K. pneumonia* bacteria the most common infection of the urinary tract infection. *K. Pneumonia* is an opportunistic pathogen, rod form, negative gram and a member of the intestinal family (2)., which is widely found in the intestines, mouth, and skin it can lead to pnemoniae, blood, surgical infections and urinary tract infections (3). The most important agents of *pneumonia* strains can be polysaccharide adhesives sideophore, lipid polysaccharides and the formation of biofilms (4). Biofilm is one of the key factors for the development of chronic diseases. Studies have shown that most *K. pneumonia* isolates from blood, wound and urine are able to produce biofilms. Bio membranes formed in vivo protect pathogens against antibiotic and host immune responses (5, 6).

Recent developments in nanotechnology, especially the ability to manufacture nanoparticles in different shapes and sizes, have caused a wide range of antimicrobial agents. The small size (<100 nm) and the ratio of the surface to the large size of the nanoparticles led to the unique chemical, thermal, mechanical and electrical properties of their massive materials and increased biological and chemical activity(7). To date,

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many nanoparticles have been actively synthesized against antimicrobial agents, such as Ag, Ni, ZnO, Au, and Cu nanoparticles. Nanoparticles kill bacterial cells through various mechanisms, including cell wall binding and cell penetration, the release of metal ions, interaction with their groups in enzymes and proteins, the accumulation of nanoparticles in the cytoplasm, and the production of ROS(8).

Most bacteria are present in the form of biofilm, which often contains diverse types that interact with each other and their environment. Bio-membranes specifically microbial aggregates and extracellular products such as extracellular polymeric materials. (9).The bacteria growth inversely on the out surface but the expression of extracellular polymers the attachment irreversible. Once bacteria are stable bacterial whip is discouraged and the bacteria multiply rapidly, leading to the development of mature biofilm. From this step the bacteria are restrict together and form a barrier that can tolerate antibiotics and supply a source of chronic systemic infection. (10). The aim of this study was to investigate the potential antibacterial and antibiofilm activities of nanoparticles against *K. pneumonia* associated with urinary tract infection.

Materials and Method

Collection bacterial strains

50 strains of (*K .pneumonia*) were collected from urine sample of patients in Baghdad / Iraq hospitals. They were grown in nutrient broth, and nutrient agar. To isolate urinary tract strains loop full of urine samples was placed in nutrient agar blood agar and agar chocolate plates and incubated at 37 ° C for one day. Individual colonies were selected the following day and were determined based on morphological characteristics, gram staining and biochemical characters (11).

Biofilm formation assay

Measurement of biofilm formation among the (*K.pneumoniae*) strains was carried out by using the tube method and tissue culture plate assay. In this tissue culture method, 100 µl (0.5 McFarland) of an overnight culture of strains was inoculated into each well of a 96-well polystyrene plate, and incubated at 37 ° C for 48 days. After incubation period, the bacteria were stained with 150 µl of crystal violet (0.1 %) for 10 min. Finally, 100 µl of acetic acid (33 % $^{v}/_{v}$) was added to each of the wells and the optical density was measured at 545 nm.

Antibiofilm activity assay of nanoparticles

Antibiofilm activity of nanoparticles (Ag, Al_2O_3 and Ni) alone and in mix with each other, against biofilm producing strains was performed by micro titer plate method. Briefly, 100 µl of bacterial suspension (0.5 McFarland) was inoculated into 96 plate wells, with different concentrations of Ag (0.031 – 2 mg ml^{-1}), Al_2O_3 (0.031 – 1 mg ml^{-1}) and Ni (0.031 – 4 mg ml^{-1}) nanoparticles and incubated at 37 °C for 48h. And read on absorbance at 545 nm (12).

Cytotoxicity test:

Both Neuro 2a cells were cultured using DMEM containing 10% fetal bovine serum (fbs) and 1% antibiotics (100 mg/ml streptomycin and 100 U/ml penicillin) and were maintained at 37□ in a humidified atmosphere with 5% CO2 They were subculture every 2 days when they reached 90% confluence by digestion with trypsin/EDTA .

MTT assay

The MTT assay was performed on Neuro 2a cells. The eight concentrations of Ag NPs (0.78 – 100 µg ml^{-1}) and Al_2O_3 NPs (1.95 – 500 µg ml^{-1}) were prepared in water by two-fold serial dilution method. 50 µl of various concentrations of Ag and Al_2O_3 NPs were added to wells containing cells, separately. Untreated cells were taken as the control. Over time, 20 µl of MTT solution at a concentration of 5 mg ml^{-1} were added to each well, and the plate was incubated for 4 hours. Afterward, the medium containing MTT was removed and 200 µl of DMSO solution was added to each well to dissolve the formazan crystals formed in living cells. Finally, after 15 min of incubation, the optical absorption of each well was read by ELISA reader at a wavelength of 570 nm. All tests were performed in triplicate and the results were reported as cell viability referred to control (Eq. 1).

$$\text{cell viability \%} = \frac{\text{OD treatment} - \text{OD blank}}{\text{OD control} - \text{OD blank}} \times 100 \qquad \text{(Eq. 1)}$$

Statistical analysis

All the experimental, data were expressed as means (means ± SD). The antimicrobial activity data were analyzed using the one-way ANOVA with IBM SPSS version 20.1.

Results

Out of 50 (*K. pneumonia*) isolate collected from the Urinary tract infection patients, were obtained. Initial identification of bacterial strains using biochemical tests, showed that 100% of the strains were negative for catalase, oxidase, and endol and positive for VP. 95% of cases were positive for urea's and citrate and 90% were positive for H2S. As showed in Fig 1

Antibiotic susceptibility testing

The Antibiotic susceptibility testing cards used to determine antibiotic susceptibility and ESBLs producing strains. Based on the antibiotic resistance profile, 9 (17.6 %) from strains were sensitive (S) and 42 (82.3 %) from strains were resistant (R). The highest and lowest resistance among *K. pneumoniae* strains was observed for ampicillin (AMP) (100 %) and tigecycline (TGC) (0 %), respectively. The piperacillin/tazobactam (TMP-SMX), cefazolin (CFZ), cefoxin (FOX), ceftazidime (CAZ), ceftriaxone (CTR), cefepime (FEP), imipenem (IPM) and amikacin (AMK) antibiotics with 82 % resistance among the strains were ranked second. It was also found that none of the bacterial strains produces ESBLs. Yang and colleagues reported that among 137 *K.pneumoniae* strains isolated urine, more than 85 % of ESBLs-producing bacteria have the ability to produce biofilm, whereas our results showed that none of the strains were ESBLs producers.

The result in the Table 1. Observe the minimum biofilm eliminating concentration (MBEC) of the mixed nanoparticles also found that Ag - Al_2O_3 NPs has the highest inhibitory effect on biofilm formation of *K. pneumoniae* strains. Treatment of *K. pneumoniae* strains with 0.5 mg ml^{-1} of Ag - Al_2O_3 NPs completely prevented the formation of biofilm in K-13 and K-36 strains. The checkerboard assay was used to evaluate the synergistic effects of mixed nanoparticles. Statistical analysis revealed that there was no significant difference in MBEC of nanoparticles against *K. pneumoniae* strains in the concentration range of 0.031 – 1 mg ml^{-1} ($p > 0.05$).

The minimum inhibitory concentration (MIC) against bacterial strains were performed using broth microdilution assay. As you can see in the Table 2, the results of the statistical analysis shows that there is a significant difference between the GI % of Ag and Ni NPs, as well as Al_2O_3 and Ni NPs in the concentration range of 0.125 – 1 mg ml^{-1} in *K. pneumoniae* strains. Significant ($p > 0.05$). The interaction between Ag – Ni and Ag – Al_2O_3 NPs was synergistic against all the *K. pneumoniae* strains. While an indifference effect was observed for Al_2O_3 - Ni NPs in *K. pneumoniae* strains.

The MTT assay was used to assess the cytotoxicity of NPs. The percentage of cell viability in the presence of Ag and Al_2O_3 NPs are shown in Fig. 2a, b, respectively. As it can be seen the cell survival is reduced, by increasing the concentration of (NP) nanoparticles in a concentration-dependent manner after 24 h. Ag NPs showed higher cytotoxicity toward Neuro-2a cells as compared to Al_2O_3 NPs, so that at a concentrations of 100 µg ml^{-1} of Ag NPs and 500 µg ml^{-1} of Al_2O_3 NPs decreased the Neuro-2a cells survival by 61 % and 36.7 %, respectively.

Table 1. The MBC values of the nanoparticles (alone and mixed) against biofilm forming strains

			Nanoparticles/(mg ml-1) alone		**Nanoparticles/(mg ml-1) mixed**	
Organisim	Ag	Al2O3	Ni	Ni – Ag	Al2O3 - Ag	Al2O3 - Ni
K. pneumoniae K-13	4	2	8	1	0. 5	1
K. pneumoniae K-33	4	2	8	0.5	1	1
K. pneumoniae K-36	4	2	8	1	0. 5	1

Table 2.The MIC values of the nanoparticles (alone and mixed) against *K. pneumoniae* strains

MIIC/(mg ml-1) Nanoparticles						
Organisim	Ag	Al2O3	Ni	Ni – Ag	Al2O3 - Ag	Al2O3 - Ni
K. pneumoniae K-13	4	2	8	0.060	0.25	2
K. pneumoniae K-33	4	2	8	0.060	0.25	2
K. pneumoniae K-36	4	2	8	0.060	0.125	2

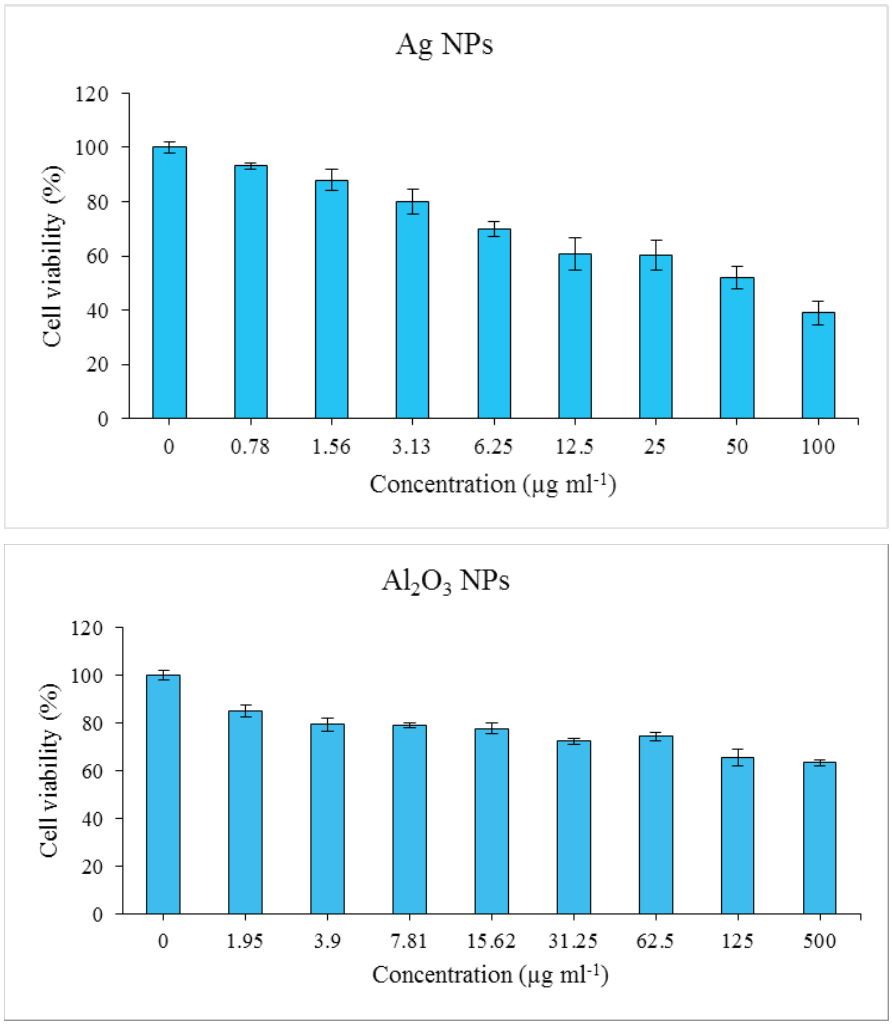

Fig. 2. Percentage of cell viability of Neuro-2a cells in presence of different concentrations of Ag NPs (a) and Al_2O_3 NPs (b)

Discussion

This study was carried out on 50 *K. pneumonia* isolates was identification of bacterial strains by biochemical tests revealed that all strains it's belong to *K. pneumonia* consistent. This is in accordance with results developed by (13). The results of biochemical tests showed that *K. pneumonia* strain is gram negative, rod-shaped, non-motile, negative gelatinase and indole, and positive catalase and urease. The strains are positive for the fermentation test of all carbohydrates Voges-Proskauer (14).

From Figure 1, it can be observed. antibiotic susceptibility cards was found a similar study by (15) reported that among 137 *K.pneumoniae* strains isolated from sputum and urine, more than 85 % of ESBLs-producing bacteria have the ability to produce biofilm, whereas our results showed that none of the strains were ESBLs producers.

Similar findings were observed the MIC and MBEC in the study conducted by (16). They evaluated the antibiofilm activity of silver nanoparticles against microorganisms using of Congo-red and tube methods, it was determined that biofilm formation in *C.neoformans* and *K.pneumoniae* bacteria was completely inhibited by tube method. Other study investigated the effects of AgNPs in different concentrations on *K.pneumoniae* biofilm; the findings demonstrated that biofilm growth was completely inhibited in 40 and 80 µg ml-1 of nanoparticles (17). The MIC results showed that the addition of thallium ions into ZnO significantly increased the bacteriostatic activity of ZnO nanoparticles against *E. coli*, *S. aureus* and *B. subtilis* strains by (18).

Many studies have also been conducted to evaluate the cytotoxic activity of Al_2O_3 NPs, but unlike other NPs, Al_2O_3 NPs have less effect for human cell lines. In one of these studies, the cytotoxicity effects of Al_2O_3 NPs on L926 and BJ cells were investigated and it was found that NPs had no significant cytotoxicity in the concentration range of 10 to 200 µg ml^{-1}, and at a concentration of 400 µg ml^{-1}, about 10% of the cell survival was reduced (**19**). Zhang et al. evaluated the impact of various NPs (ZnO, TiO_2, SiO_2 and Al_2O_3) on HFL1 cells. The findings showed that Al_2O_3 NPs had the least effect on cellular survival, and a reduction of about 10% of cell viability at a concentration of 250 µg ml^{-1} was observed (20).

Conclusions

In this study we have investigated the antimicrobial activity of different nanoparticles against urinary tract infections caused by *K. pneumonia*. The nanoparticles could be used as an effective anti-microorganism agent for the treatment of urinary tract infections caused by *K. pneumonia.* However, Ag-Ni NPs revealed more efficacy than other mixed NPs. On the other hand, Ag NPs indicated higher cytotoxicity against Neuro-2a cells as compared to Al_2O_3 NPs. It seems that the mentioned NPs can be a good candidate for the treatment of bacterial infections and the removal of antibiotic-resistant strains

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