Antibacterial action of AgNPs produced from different isolates of Gram positive and Gram-negative bacteria on biofilm of Klebsiella pneumoniae isolated from RTI

Article · July 2023

DOI: 10.51248/.v43i3

3 authors, including:

Suaad Ali Ahmed
Baghdad University College of Science
11 PUBLICATIONS **16** CITATIONS

SEE PROFILE

Enass Ghassan Sweedan
University of Baghdad
30 PUBLICATIONS **59** CITATIONS

SEE PROFILE

Research article

Antibacterial action of AgNPs produced from different isolates of Gram positive and Gram-negative bacteria on biofilm of *Klebsiella pneumoniae* isolated from RTI

Suaad Ali Ahmed[1], Hussam Mahmood Hasan[2,] Enass Ghassan Sweedan[3]

[1,2,3]Department of Biology, College of Science, University of Baghdad, Baghdad-Iraq

(Received: May 2023 Revised: May 2023 Accepted: June 2023)

Corresponding author: **Suaad Ali Ahmed**. Email: suaadbio76@gmail.com

ABSTRACT

Introduction and Aim: *Klebsiella pneumoniae* is a Gram-negative bacterium responsible for a wide range of infections, including respiratory tract infections (RTIs). This research was aimed to study the antibacterial and anti-biofilm effect of AgNPs produced by Gram positive and negative bacteria on RTIs associated with *K. pneumoniae.*

Materials and Methods: The biofilm formation of *K. pneumoniae* was determined by tube method qualitatively from select bacterial species characterized by UV-Visible spectroscopy. The antibacterial susceptibility of the bacteria AgNPs was tested for their antibacterial and antibiofilm activity on a clinical isolate of *K. pneumoniae*.

Results: *K. pneumoniae* isolated from RTIs were strong biofilm producers. The antibacterial activity of AgNPs synthesized from bacterial spp in this study had good antibacterial activity against *K. pneumoniae*. *P. aeruginosa* and *P. mirabilis* AgNPs had the strongest anti-biofilm effect, with 84% and 83%, respectively, while *A. baumanii*'s AgNPs had the lowest (79%). AgNPs of *P. aeruginosa* and *P. luteola* showed the highest (80%) anti-biofilm action against the development of pre- and post-mature biofilms formed by *K. pneumoniae*, while AgNPs from *S. mitis* exhibited the lowest levels (69%).

Conclusion: AgNPs generated by Gram positive and Gram-negative bacteria, when exposed to *K. pneumoniae* isolated from RTIs had a good antibacterial impact and inhibited the formation of biofilm by *K. pneumonia* and hence could be used as an antibacterial agent against *K. pneumoniae* infecting the respiratory tract.

Key words: *Klebsiella pneumoniae*; silver nanoparticles (AgNPs); biofilm; anti-biofilm; Gram positive; Gram negative.

INTRODUCTION

Klebsiella pneumoniae, an Enterobacteriaceae family member, is a Gram-negative, encapsulated opportunistic pathogen that causes a wide range of infections and is a major concern to public health globally (1). *K. pneumoniae* can cause a number of infections, such respiratory, blood and urinary tract infections (1). *K. pneumoniae* associated with severe hospital- and community-acquired infections are often multidrug resistance and hyper virulent (2). *K. pneumoniae* that colonize the upper part of larynx, can gain entry into blood causing serious infections such as bacteremia, septicemia, and pneumonia, which could be transmitted via ventilators in hospitals (3, 4). Among the several virulent factors associated with this pathogen, the most important is its ability to form biofilms and to being resistant to a wide range of antibiotic groups and antimicrobial agents (5, 6). Infection by *K. pneumoniae* is difficult to treat, as these infecting strains are often multidrug resistance (7).

Silver nanoparticles (AgNPs) are metallic nanoparticles that have been used in several biomedical applications, including nanomedicine, cancer and therapy (7, 8). Due to its unique properties, AgNPs have also found applications as anti-bacterial, anti-fungal, anti-viral and anti-inflammatory agents (7, 9). Recent studies have reported that AgNPs could be used as an alternative to antibiotics that can combat bacterial antibiotic resistance (8). The bactericidal effect of nanoparticles has been demonstrated on several bacterial multidrug resistant pathogens, including *Escherichia coli* and *Staphylococcus aureus* (10-12). One of the mechanisms involved in bacterial pathogenicity is the formation of biofilms which are aggregates of bacterial cells held together by a self-produced matrix that facilitates its survival in adverse conditions and niches (13). Several studies have studied the effect of AgNPs on biofilm formation and reported it to be an efficient anti-biofilm agent (7, 14, 15). Hence the aim of this study was to synthesize AgNPs from pathogenic Gram positive and negative bacterial isolates and analyze the effect of the generated AgNPs on *K. pneumoniae* pre- and post-biofilm formation.

MATERIALS AND METHODS

Isolation and identification of *K. pneumonia*e

Fifty sputum specimens from respiratory tract infections were collected from Iraqi patients hospitalized in Baghdad. All specimens were sub-cultured onto the culture medium -MacConkey agar and blood agar, and incubated overnight at 37°C. The isolates grown were subjected to primary identification based on phenotypic and biochemical assays as described previously (9, 16) and further validated by the VITEK-2 compact system (bioMérieux, France).

Among the isolates, Gram negative (*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas luteola, Acinetobacter baumanii*) and Gram positive (*Staphylococcus aureus, Streptococcus mitis, Streptococcus salivarius, Streptococcus pyogenes, Enterococcus faecalis*) were obtained from the Central Laboratory of Biology Department.

AgNPs synthesis and characterization

The AgNPs were synthesized as described by (10, 17). Bacterial strains grown in Brain heart medium were harvested by centrifugation at 6000 rpm/ 20 min. The free cell culture was mixed with $AgNO_3$ (10 mM) and incubated at 30°C for 24 hr. in dark (4). AgNPs were purified by centrifugation (10). The synthesized AgNPs were characterized by spectroscopy as mentioned by Zhang *et al*., (7).

AgNPs antibacterial activity assay

The antibacterial susceptibility of AgNPs synthesized from Gram negative (*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas. luteola, Acinetobacter baumanii*) and Gram positive (*Staphylococcus aureus, Streptococcus mitis, Streptococcus salivarius, Streptococcus pyogenes, Enterococcus faecalis*) against *K. pneumoniae* was determined by wells method (17). Each of the isolate was cultured overnight in nutrient broth, to obtain a turbidity of approximately 1.5×10^8 cells/ml and then plated onto Muller-Hinton agar medium. Wells were made on plates, and 100 µl of crude AgNPs was filled in by micropipette, then plates incubated overnight at 37° C, followed by measurement of the inhibition zones developed.

Formation of biofilm assay

The biofilm formation of *Klebsiella pneumoniae* was determined by the qualitative tube method (18). A 50 µl of (*Klebsiella pneumoniae)* in brain heart infusion broth was prepared in the turbidity mentioned above. After incubation, the culture was washed twice with a PBS buffer. Then tubes were stained with crystal violet (0.1%) for 30 min. The assay included a negative control. The tubes were left at room temperature and the biofilm formation ability was detected by the appearance of a blue layer.

Anti-biofilm activity of AgNP particles

Each bacterial AgNP was assayed for its anti-biofilm effect on pre-mature biofilm of *K. pneumoniae* by the microtiter plate assay (17). To each well in the plate 100 µl of brain heart infusion broth was added, followed by 100 µl of respective AgNP and 10 µl of *K. pneumoniae* culture grown overnight. The plates were incubated at 37°C for 24 hr, following which the contents were washed three times with PBS to remove excess cells. The biofilms formed were stained with crystal violet (0.1%) for 10 min, washed with water and air dried. In order to quantify the number of cells in biofilms, 200 µl of 95% ethanol was added into each well, and absorbance read at 620 nm using an ELISA reader. For AgNPs effect on post-mature biofilm of *K. pneumoniae,* the same steps that mentioned above were used with substitution adding of AgNPs after 24 hr. incubation at 37° C. Negative and positive controls were also employed. Biofilm inhibition percent was calculated using the formula:

Biofilm inhibition percent = $(O.D_{(Control)} - O.D_{(Test)} / O.D_{(Control)}) \times 100\%$

RESULTS

Isolation and identification of *K. pneumoniae*

Twelve *K. pneumoniae* strains were isolated from fifty respiratory tract infection patients hospitalized in Baghdad city hospital. The bacteria were identified by their pink colonies on MacConkey agar. Biochemically these strains were lactose fermentation, Simmon citrate and indole test positive.

AgNPs characterization

The AgNPs were characterized by change in color to dark brown was conceded as indicator for forming nanoparticles, as shown in Fig. 1. The AgNPs were characterized by UV-visible spectroscopy. The absorbances of particles were measured between 300-700 nm. The absorbance peak for the Gram-positive *E. faecalis, S. aureus, S. pyogenes, S. salivarius, S. mitis* strains were seen at values 450 nm, 430 nm, 460 nm, 430 nm, 420 nm respectively, while for the Gram-negative *A. baumanii, P. mirabilis, E. coli, P. luteola, P. aeruginosa* strains the absorbance peaks observed were at 435 nm, 460 nm, 440 nm, 450 nm, 470 nm respectively.

Antibacterial activity assay of AgNPs

The data showed a clear inhibitory effect of AgNPs against *K. pneumoniae* strain that was used in this study. The synthesized Gram positive and negative bacterial AgNPs nanoparticles exhibited a good antibacterial effect, as shows in Fig. 3. The highest inhibition zone (30 mm) was exhibited by AgNP from *P. aeruginosa*, followed by *S. aureus* and *E. faecalis*, 29 mm and 27 mm respectively. Then *E. coli* and *S. pyogenes* AgNPs showed the same inhibition zone of 26 mm. The remaining bacterial AgNPs showed inhibition zones of diameters ranging from 25-19 mm against *K. pneumoniae* (Fig.2).

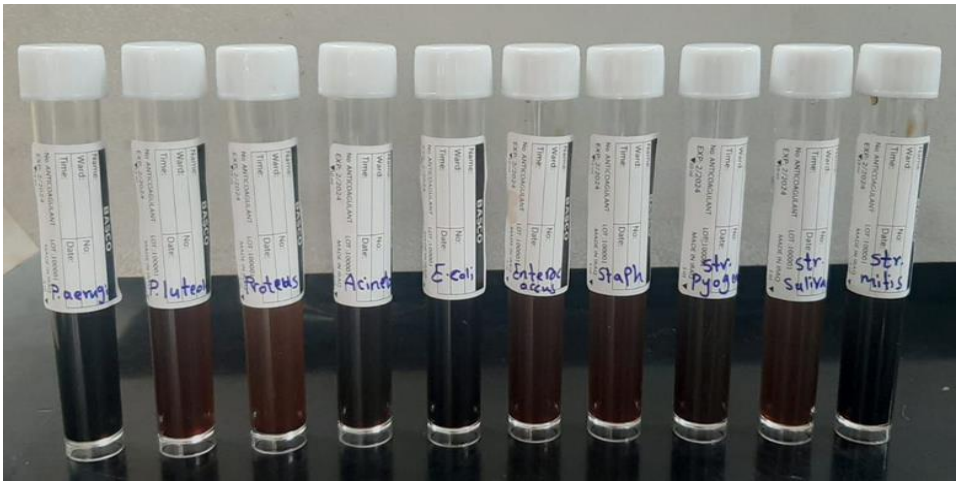

Fig. 1: Synthesis of silver nanoparticles by bacterial species used in the study

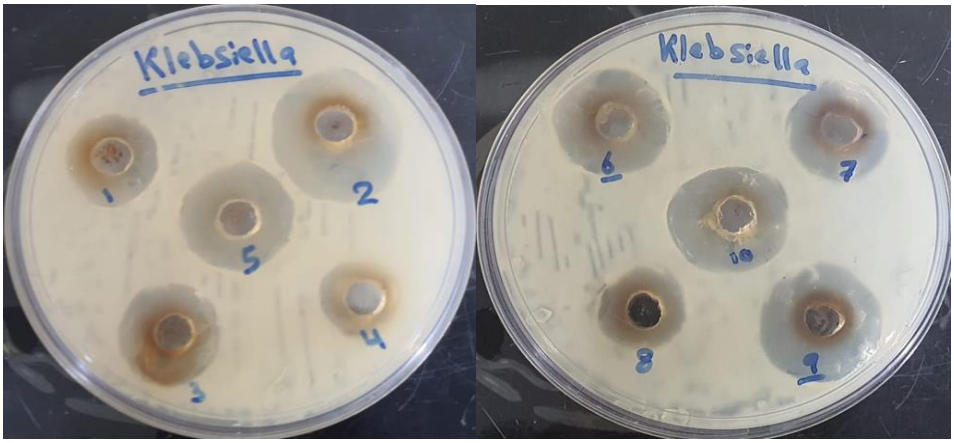

Fig. 2: Antibacterial effect of AgNPs produced from Gram +ve and Gram-ve against *K. pneumoniae*
1. *E. faecalis* **2.** *S. aureus* **3.** *S. pyogenes* **4.** *S. salivarus* **5.** *S. mitis* **6.** *A. baumanii* **7.** *Proteus mirabilis* **8.** *E. coli* **9.** *P. luteola* **10.** *P. aeruginosa*

Biofilm production and anti-biofilm activity of AgNPs

In this study, AgNPs synthesized from the Gram positive and Gram-negative bacterial strains isolated from respiratory tract infection patients were investigated for their anti-biofilm activity against a strong biofilm forming *K. pneumoniae* strain. The results showed the bacterial AgNPs to effective in inhibiting premature and post mature biofilm formation by *K. pneumoniae*. The biofilm inhibition percentage of each AgNP on pre- and post- mature biofilm by *K. pneumoniae* is presented in Table 1. As seen the highest anti-biofilm effect on pre mature biofilm was by *P. aeruginosa* (84%) *and P.mirabilis* (83%) AgNP, while the lowest was from *A. baumanii* (79%). Similarly, AgNPs showed anti-biofilm activity against post mature biofilm formation, with the highest biofilm inhibition exhibited by AgNP from *P. aeruginosa* (80%) and *P. luteola* (80%), and the lowest by AgNP from *S. mitis* (69%) (Table 1).

Table 1: The percent of biofilm formation inhibition on *K. pneumoniae* premature biofilm and post mature biofilm treated with AgNPs

AgNPs Particles from bacteria		***K. pneumoniae* biofilm inhibition (%)**	
		Biofilm (%) premature biofilm with AgNPs	**Biofilm (%) post mature biofilm with AgNPs**
Gram positive	*S. aureus*	81%	74%
	S. mitis	81%	69%
	S. pyogenes	80%	79%
	E. faecalis	80%	70%
	S. salivarius	80%	79%
Gram negative	*P. aeruginosa*	84%	80%
	P. luteola	81%	80%
	P. mirabilis	83%	78%
	E. coli	81%	71%
	A. baumanii	79%	78%

DISCUSSION

Silver nanoparticles (AgNPs) extracted from plants, fungi and bacteria have been shown to have numerous biomedical applications, and are particularly known for their anti-bacterial and anti-biofilm activity for many pathogens (19, 20). The antibacterial activity of the AgNPs which synthesis from banana peel extract (BPE) against multidrug resistance (MDR) bacteria were showed a considerable effect against MDR isolates (*Escherichia coli, Salmonella typhi, Enterobacter aerogenes, Citrobacter freundii and Hafnia alvei, Proteus mirabilis, Pseudomonas aeruginosa and Klebsiella pneumoniae*; 21). In this study, AgNPs derived from distinct Gram positive and Gram negative bacterial strains isolated from respiratory tract patients were tested for their antibacterial and anti-biofilm activity against a multi-drug resistant and strong biofilm forming *K. pneumoniae* strain. Our studies showed the AgNPs of these bacterial strains to exhibit strong antibacterial as well as anti-biofilm effect against *K. pneumoniae* tested. Our results are in line with previous studies which have demonstrated the antibacterial effects of AgNPs on growth of Gram-negative *K. pneumoniae, Psuedomonas aruginosa, Proteus mirabilis, Escherichia coli* and *Acinetobacter baumannii* strains (19, 22 23). Chemically synthesized AgNPs were demonstrated to strongly inhibit biofilm formation by the pathogens *E. coli*, *P. aeruginosa* and *Serratia proteamaculans* at concentrations of 4–20 μg/ml (23). Studies into the molecular mechanisms of AgNPs action have revealed that these nanoparticles severely inhibit bacterial multiplication and biofilm formation by downregulating the transcription level of key virulence and biofilm-related genes (24, 25). Furthermore, a recent study found drug resistance genes carried by a clinical isolate of *K. pneumoniae* to be linked to biofilm gene expression. Thus, further studies are needed to better understand the mechanisms by which the AgNPs isolated by bacterial species in this study interfere with pre- and post-mature biofilm activity of *K. pneumonia* (26).

CONCLUSION

AgNPs synthesized from Gram positive and negative bacteria exhibited good antibacterial effects against *K. pneumonia*e and strongly inhibited their biofilm formation. Hence these AgNPs could be used as an antibacterial agent and anti- biofilm action against *K. pneumoniae* infecting the respiratory tract.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

REFERENCES

1. Effah, C.Y., Sun, T., Liu, S., Wu, Y. *Klebsiella pneumoniae*: an increasing threat to public health. Ann Clin. Microbiol. Antimicro. 2020; 19(1):1-9.
2. Dong, N., Yang, X., Chan, E.W., Zhang, R., Chen, S. *Klebsiella* species: Taxonomy, hyper virulence, and multidrug resistance. eBioMedicine. 2022;79: 103998.
3. Seifi, K., Kazemian, H., Heidari, H., Rezagholizadeh, F., Saee, Y., Shirvani, F., *et al.*, Evaluation of biofilm formation among *Klebsiella pneumoniae* isolates and molecular characterization by ERIC-PCR. Jundishapur J Microbiol. 2016; 9(1): e30682.
4. Ochonska, D., Scibik, L., Brzychczy-Wloch, M. Biofilm Formation of Clinical *Klebsiella pneumoniae* Strains Isolated from Tracheostomy Tubes and Their Association with Antimicrobial Resistance, Virulence and Genetic Diversity. Pathogens.2021; 10(10):1345.
5. Nirwati, H., Sinanjung, K., Fahrunissa, F., Wijaya, F., Napitupulu, S., Hati, V.P. et al., Biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. BMC Proceedings.2019; 13(11):1-8.
6. Malachowa, N., Kobayashi, S.D., Porter, A.R., Freedman, B., Hanley, P.W., Lovaglio, J., *et al.*, Vaccine protection against multidrug resistant *Klebsiella pneumoniae* in a nonhuman primate model of severe lower respiratory tract infection. mBio.2019; 10: e02994-19.
7. Zhang, X.F., Liu, Z.G., Shen, W., Gurunathan, S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. Int. J Mol. Sci. 2016; 17(9):1534.
8. Das, S., Sharma, R., Kalyani, M.I., Nath, N., Kalita, M.C., Shukla, S. Sunlight driven biosynthesis of silver nanoparticles using aqueous stem extract of Tinospora sinensis (Lour.) Merr. and evaluation of its catalytic and antibacterial activity. Biomedicine. 2020 Nov 9;40(3):301-308.
9. Bruna, T., Maldonado-Bravo, F., Jara, P., Caro, N. Silver nanoparticles and their antibacterial applications. Int. J. Mol. Sci. 2021; 22(13): 7202.
10. Siddiqi, K.S., Husen, A., Rao, R.A. A review on biosynthesis of silver nanoparticles and their biocidal properties. J. Nanobiotechnol. 2018; 16:1-28.
11. Al-Saidi, M.H., Al-Bana, R.J.A., Hassan, E., Al-Rubaii, B.A.L. Extraction and characterization of nickel oxide nanoparticles from Hibiscus plant using green technology and study of its antibacterial activity. Biomedicine (India). 2022; 42(6):1290-1295.
12. Saleh, T.H., Hashim, S.T., Malik, S.N., AL-Rubaii, B.A.L. Down-regulation of f*lil* gene expression by Ag nanoparticles and TiO2 nanoparticles in pragmatic clinical isolates of *Proteus mirabilis* and *Proteus vulgaris* from urinary tract infection. Nano Biomedicine and Engineering. 2019; 11(4):321-332.
13. Shahverdi, A.R., Fakhimi, A., Shahverdi, H.R., Minaian, S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. Nanomedicine. 2007; 3:168-171.
14. Kostakioti, M., Hadjifrangiskou, M., Hultgren, S. J. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the post antibiotic era. Cold Spring Harb Perspect. Med. 2013 ;3(4): a010306.
15. Srikanth R., Thupurani M.K., Rao H., Surekha C. Anti-biofilm activity and time kill kinetic effects of Salacia oblanga wall leaf and root extracts against clinical multi-drug resistance bacteria. Biomedicine. 2020 Nov 9;40(3):347-352.
16. Riedel, S., Morse, S.A., Mietzner, T.A., Miller, S. Jawetz Melnick & Adelbergs Medical Microbiology 28 E. 2019. McGraw Hill Professional.
17. Ahmed, S.A. Hasan, H. M. Comparison in Synergetic Effect for Silver Nanoparticles that Produced from *P. aeruginosa and P. luteola* with Oil Extract of *Cymbopogon Citraus* on Different Types of Pathogenic Bacteria. Indian Journal of Forensic Medicine and Toxicology. 2021;15(4): 2589-2595.
18. Obaid, H.H., Khalaf, Z.Z., Tawfeeq, H.K., Sabri, R.T., Abdul-Jabbar Z. Antimicrobial Effect of *Rheum ribes* and

Tio2 Nps on Bacterial Biofilm in *Escherichia coli*. IOSR Journal of Pharmacy and Biological Sciences.2017; 12 (3): 14-20.

19. Ahmed, S.A., Hasan, H.M., Ahmed, E.F. Silver nanoparticles biosynthesis and their antimicrobial activity against wild and mutant isolates of different G-ve bacterial types. Bioscience Research. 2018; 15(4):2997-3005.
20. Saleh R.F., Gaidan A.M. Biosynthesis and characterization of silver nanoparticles using Cinnamomum zeylanicum extract and a study of antibacterial effect against multi-drug resistance Gram-negative bacteria. Biomedicine. 2021;41(2):249-255.
21. Tawfeeq, S. M., Maaroof, M. N., Al-Ogaidi, I. Synergistic effect of biosynthesized silver nanoparticles with antibiotics against multi-drug resistance bacteria isolated from children with diarrhea under five years. Iraqi Journal of Science. 2022; 58(1A):14-52.
22. Saleh, G.M. Green Synthesis Concept of Nanoparticles from Environmental Bacteria and Their Effects on Pathogenic Bacteria. Iraqi Journal of Science. 2020; 61(6):1289-1297.
23. Radzig, M.A., Nadtochenko, V.A., Koksharova, O.A., Kiwi, J., Lipasova, V.A., Khmel, I.A. Antibacterial effects of silver nanoparticles on Gram-negative bacteria: influence on the growth and biofilms formation, mechanisms of action. Colloids Surf B Biointerfaces. 2013; 102:300-306.
24. Hetta, H.F., Al-Kadmy, I., Khazaal, S.S., Abbas, S., Suhail, A., El-Mokhtar, M.A., *et al*., Antibiofilm and antivirulence potential of silver nanoparticles against multidrug-resistant *Acinetobacter baumannii*. Sci Rep. 2021; 11: 1-11.
25. Sweedan, E.G., Majeed, S.M.A. Effects of silver nanoparticles synthesized from phenolic extract of Agaricus bisporus against pathogenic bacteria and yeasts. Nano Biomedicine and Engineering. 2023; 15(1):32-41.
26. Joshi, A.S., Singh, P., Mijakovic, I. Interactions of gold and silver nanoparticles with bacterial biofilms: Molecular interactions behind inhibition and resistance. Int. J. Mol. Sci. 2020; 21: 7658.