RESEARCH ARTICLE

Investigating the possibility of treating Serratia marcescens with an antibiotic-resistant mixture containing nanoparticles using AgNPs as a new type of antimicrobial: An experimental study

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Abstract

Objective: To explore the possibility of treating serratia marcescens with an antibiotic-resistant mixture containing silver nanoparticles.

Method: The experimental study was conducted at the Bacteriology Laboratory of Ibn Al-Baladi Hospital, Baghdad, Iraq, from December 2021 to April 2022, and comprised human urine samples, a wound sample from local chickens, and respiratory secretions from pigeons. The isolates were kept on a brain-heart infusion medium with glycerol. Their response to antibiotics with different concentrations of 9% and 7% silver nanoparticles were checked. To optimise factors for the effect of silver nanoparticles, incubation time, temperature and silver nitrate concentration were the three parameters used. Disc diffusion method was used to evaluate the antibacterial activity of silver nitrate against serratia. The inhibitory zone developed was measured. Clinical and Laboratory Standards Institute's guidelines were followed.

Result: Optimal silver nitrate concentration was 9%, and 37°C temperature and incubation time 24h was needed for silver nanoparticle production. Silver nanoparticle had 100% antibacterial activity.

Conclusion: Nanoparticles were found to have the potential to become a viable therapeutic option.

Keywords: Silver nitrate, *Serratia marcescens*, Nanoparticles, Temperature, Biocompatible, Silver, Anti-infective.

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Introduction

Serratia (S.) marcescens belongs to the enterobacteriaceae family and is a member of the serratia genus. There are now 14 species of serratia in genus Serratia, with eight of them linked to human sickness.[1] The most well-known of the eight species linked to clinical illness are *S. marcescens, S. liquefactions* and *S. odorifera*.[1,2] The spread of multi-drug resistance (MDR) among clinically significant bacterial species highlights the urgent need for new antibacterial therapies.[3,4] *S. marcescens* isolates have been shown to interact in cell signalling mechanisms involved in biofilm development.[5] If further research confirms biofilm's harmful involvement in *S. marcescens*, it is possible that it is related to other aspects of this opportunistic illness, such as adherence, colonisation, and antibiotic resistance. *Enterobacteriaceae* have exhibited an extraordinary ability to acquire, transfer and modify the expression of numerous antibiotic resistance genes over the last two decades.[6] The reasons for the synergistic interaction are unknown. However, it has recently been shown that the ampicillin-induced over-expression of AmpC β-Lactamases goes along with changes in outer membrane profiles, which, in turn, can foster the efficiency of phage infection[7]. Decreased membrane permeability and active efflux of toxic substances that limit the effectiveness of antimicrobials are referred to as "intrinsic resistance". Acquired resistance is most frequently brought on by plasmid horizontal gene transfer, or gene mutations. Phenotypic resistance, also referred to as adaptive resistance, is connected to a rapid transcriptome response to environmental or stressful stimuli.[8] In recent years, there has been much interest in the biosynthesis of nanomaterials. If fully employed, biological synthesis might give an edge over chemical approaches in terms of increased productivity and cheaper costs. In recent years, a new dimension of metal-microbial interaction has been researched for the production of metal nanoparticles, such as gold, silver, cadmium, zirconia and silica titanium.[9] Synthesis of metal nanoparticles from bacteria, yeasts, fungi and other biological sources has been reported, and the same has been applied to a variety of fields, such as drug delivery, biosensors, bioimaging, antimicrobial activity, food preservation and so on.[10]

The current study was planned explore the possibility of treating *S. marcescens* with an antibiotic-resistant mixture containing silver nanoparticles (AGNPs).

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Materials and Methods

The Experimental study was conducted at the Bacteriology Laboratory of Ibn Al-Baladi Hospital, Baghdad, Iraq, from December 2021 to April 2022, and comprised human urine samples, a wound sample from local chickens, and respiratory secretions from pigeons (Table 1). *S. marcescens* strains were taken from a previous study[11] done at the same centre. These isolates were kept on a brain-heart infusion medium with glycerol, and were registered with the National Centre for Biotechnology Information (NCBI) (MZ395963.1, MZ395964.1; MZ395967.1, MZ395968.1; and MZ395969.1, MZ395970.1).

Silver nitrate (AgNO3) (Sigma Aldrich, St. Louis, USA) and Muller Hinton agar (HI media, India) were used, and antibiotic susceptibility test for various classes of antibiotics, including augmentin (AUG) (amoxicillin and clavulanic acid), amikacin (AK) and norfloxacin (NOR) was carried out by modified Kirby-Bauer disk diffusion method[12] in the light of Clinical Laboratory Standard Institute (CLSI) recommendations.[13]

In 100ml of deionized distilled water (DW), 16.98g of AgNO3 was dissolved. Then, 1ml of the prepared solution was added to another 1000ml of DW to make a 1mM solution.[14]

Silver nanoparticles (AgNPs) were prepared from alcoholic extracts with some modifications[15], and 5ml were distilled into a 95ml molar amyl AgNO3 solution under ultrasonic conditions, with an ultrasonic power of 100W and a frequency of 42kHz. After 20min of sonication, the solution was stirred at 800rpm at 25°C for 30min, and the samples were then stored in dark bottles at 25°C for 24h. The final samples were stored in dark bottles, and within 5 days, the colour change of the solution was observed, indicating the formation of AGNPs. The aqueous Ag+ ions were reduced during exposure to the culture supernatant of *S. marcescens*. The colour of the reaction solution turned from yellow to brown due to the formation of NPs.

Disc diffusion method was adopted to evaluate the antibacterial activity of AgNPs prepared against serratia according to the use of filter paper that was immersed in AgNPs for 2min before culturing them on plates to determine the inhibition zone and then these plates were incubated for 24h at 37°C. The inhibitory zone developed after 24 h and was measured, quantified and compared.[16]

Results

The inhibition zone, indicating the area on the agar medium where bacterial growth stopped, was clearly visible (Figure 1). This test was performed on three isolates using three different antibacterial drugs. Antibiotic susceptibility testing findings were noted (Table 2).

AgNPs could inhibit *S. marcescens* at different concentrations, and the highest inhibition area was at 9% concentration (Table 3; Figures 2-4).

Table-1: Types and sources of Serratia marcescens isolates.

NO.	Type of samples	Sources of samples	isolates
1	Urine	Human	8H
2	Wound	Local chicken	6W
3	Respiratory secretion	pigeon	4R

Table-2: Results of antibiotic susceptibility testing.

Antibiotic	Concentration (µcg/ disc)	Susceptible	Intermediate	Resistant
Amoxicillin / Clavulanic acid (Augmentin)	AUG (30)	_____	_____	6mm
Amikacin	AK (25)	_____	_____	15mm
Norfloxacin	NOR (10)	27mm	_____	_____

Table-3: The effect of antibiotics with silver nanoparticles on Serratia marcescens.

Concentration (µcg/ disc)	8H	6W	4R
9% silver nanoparticles	12mm	13mm	11mm
Amoxicillin / Clavulanic acid [Augmentin (AUG 30)] with 9% silver nanoparticles	14mm	16mm	17mm
7% silver nanoparticles	10mm	11mm	12mm
Amoxicillin / Clavulanic acid [Augmentin (AUG 30)] with 7% silver nanoparticles	12mm	14mm	15mm

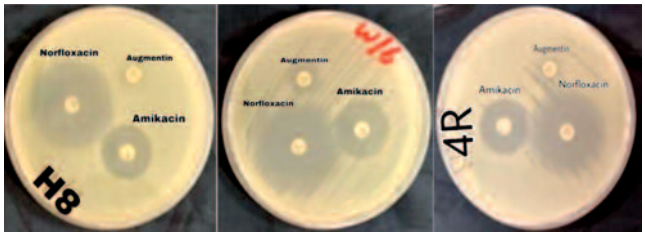

Figure 1: Antibiotic susceptibility testing of birds serratia marcescens isolates (4R, W6, 8H) on Mueller Hinton agar.

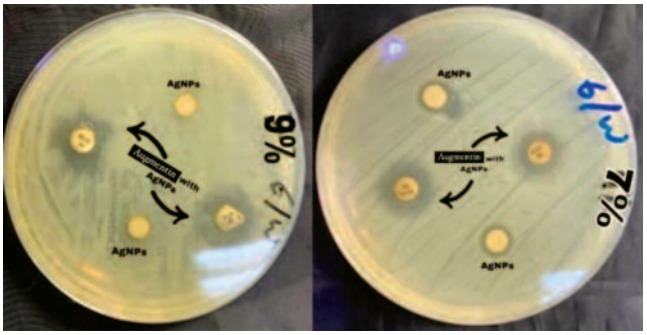

Figure 2: Comparison of clear visible area of silver nanoparticle (AgNPs) agent concentrations (9%, 7%) with a mixture of Amoxicillin / Clavulanic acid and AgNPs for serratia marcescens inhibition (6W).

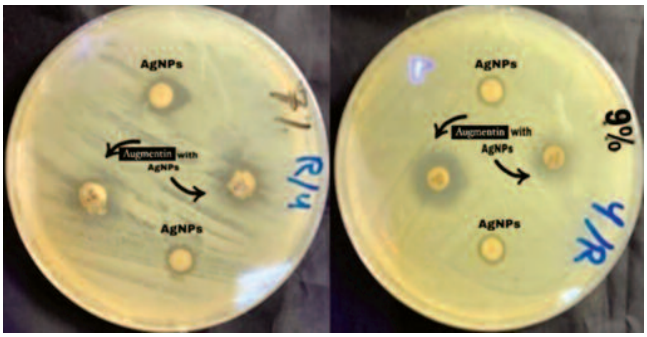

Figure 3: Comparison of clear visible area of silver nanoparticles (AgNPs) agent concentrations (9%, 7%) with a mixture of Amoxicillin / Clavulanic acid and AgNP concentrations (9%, 7%) for serratia marcescens inhibition (4R).

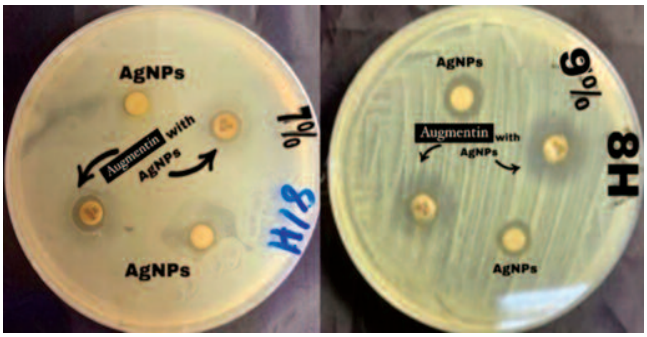

Figure 4: Comparison of clear visible area of silver nanoparticles (AgNPs) agent concentrations (9%, 7%) with a mixture of Amoxicillin / Clavulanic acid and AgNP concentrations (9%, 7%) for serratia marcescens inhibition (8H).

Discussion

AgNPs exhibited antibacterial action against gram-negative bacteria. Because the disk diffusion test was the preliminary screening tool, a further assessment in identifying the antibacterial activity of AgNPs is required[17]. Resistance to ampicillin, amoxicillin, amoxicillin-clavulanate, and cefuroxime has previously been documented in *S. marcescens*.[18] The antibacterial action of AgNPs is owing to their tiny size and high surface area-to-volume ratio.[19] Using dose-dependent growth kinetics of bacterial strains, the relative rate and extent of antibacterial activity of AgNPs can be determined. Three representative bacterial strains' growth profiles after being treated with 10nm AgNPs were part of the current study. When compared to the negative control, the addition of AgNPs affected the growth kinetics of all bacterial strains. As the concentration of AgNPs increased, bacterial growth was inhibited, which is in line with literature.[20] Studies on the mechanism of action of NPs-antibiotic combinations have shown that the increase in antibacterial activity may be owing to their chemical interaction, but the underlying chemical mechanism of the action, whether synergistic or antagonistic, is yet unknown.[21] Recent research indicates that silver cation (Ag+) is more likely to be the agent causing cell death.[22] Ag+ increases the bactericidal activity of antibiotics by increasing the generation of reactive oxygen species, which causes the bacterial cell to die. Antibiotics combined with AgNPs are effective against resistant bacteria.[23] The particle size of AgNPs influences their bactericidal effects; the greater is the antibacterial efficacy, the smaller are the particles.[19]

Further studies are required to validate the current findings and to investigate the effects of AgNPs on various bacteria in order to decrease their microbial pathogenicity.

Conclusion

AgNPs might be a beneficial synergistic agent to develop more effective antibacterial treatments against MDR bacterial strains.

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Conflict of Interest: None.

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References

1. Mahlen SD. Serratia infections: from military experiments to current practice. Clin Microbiol Rev 2011;24:755-91. doi: 10.1128/CMR.00017-11.
2. Farmer JJ III. Enterobacteriaceae: Introduction and Identification. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of Clinical Microbiology. Washington, DC: American Society for Microbiology Press, 1995: 438-449.
3. Aslam S, Courtwright AM, Koval C, Lehman SM, Morales S, Furr CL, et al. Early clinical experience of bacteriophage therapy in 3 lung transplant recipients. Am J Transplant 2019;19:2631-9. doi: 10.1111/ajt.15503.
4. Verbeken G, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, et al. Call for a dedicated European legal framework for bacteriophage therapy. Arch Immunol Ther Exp (Warsz) 2014;62:117-29. doi: 10.1007/s00005-014-0269-y.
5. Lin CS, Horng JT, Yang CH, Tsai YH, Su LH, Wei CF, et al. RssAB-FlhDC-ShlBA as a major pathogenesis pathway in Serratia marcescens. Infect Immun 2010;78:4870-81. doi: 10.1128/IAI.00661-10.
6. Shanks RM, Stella NA, Kalivoda EJ, Doe MR, O'Dee DM, Lathrop KL, et al. Serratia marcescens OxyR homolog mediates surface attachment and biofilm formation. J Bacteriol 2007;189:7262-72. doi: 10.1128/JB.00859-07.
7. Wang S, Yin B, Yu L, Dang M, Guo Z, Yan G, et al. Overexpression of AmpC Promotes Bacteriophage Lysis of Ampicillin-Resistant Escherichia coli. Front Microbiol 2020;10:e2973. doi: 10.3389/fmicb.2019.02973.
8. Hasan CM, Dutta D, Nguyen ANT. Revisiting Antibiotic Resistance: Mechanistic Foundations to Evolutionary Outlook. Antibiotics (Basel) 2021;11:40. doi: 10.3390/antibiotics11010040.
9. Fayaz AM, Girilal M, Mahdy SA, Somsundar SS, Venkatesan R, Kalaichelvan PT. Vancomycin bound biogenic gold nanoparticles: a different perspective for development of anti VRSA agents. Process Biochem 2011;46:636-41. Doi: 10.1016/j.procbio.2010.11.001.
10. Bansal V, Rautaray D, Ahmad A, Sastry M. Biosynthesis of zirconia nanoparticles using the fungus Fusarium oxysporum. J Mater Chem 2004;14:3303-5.

11. Kashash RR, Abdul-Kareem IQ. Molecular Identification of in Humans and Serratia marcescens Birds in Baghdad Province, Iraq. Indian J Ecol 2022;49(Special Issue):502-8.
12. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
13. Clinical and Laboratory Standards Institute (CLSI). CLSI supplement M100: Performance Standards for Antimicrobial Susceptibility Testing, 32nd ed. Wayne, PA: Clinical and Laboratory Standards Institute, 2022.
14. Al-Nuaimi MA, Hamdan NT, Abdalraheem E, Aljanabi ZZ. Biodegradation of Malathion pesticide by silver bio-nanoparticles of Bacillus licheniformis extracts Res on Crops 2019;20(Special Issue):s79–84. DOI: 10.31830/2348-7542.2019.138
15. Al-Nuaimi MA. Effect of Metarhizium anisopliae and silver nanoparticles manufactured by it on the development of Trogoderma granarium. [PhD thesis] Baghdad, Iraq: College of Science for Women University of Baghdad; 2018. [Online] [Cited 2023 July 23]. Available from URL: https://uobaghdad.academia.edu/drMarwahALNuaimi
16. Abbas SZ, Hussain K, Hussain Z, Ali R, Abbas T. Anti-Bacterial Activity of Different Soaps Available in Local Market of Rawalpindi (Pakistan) against Daily Encountered Bacteria. Pharm Anal Acta 2016;7:e522. doi:10.4172/2153-2435.1000522.
17. Burt S. Essential oils: their antibacterial properties and potential applications in foods--a review. Int J Food Microbiol 2004;94:223-53. doi: 10.1016/j.ijfoodmicro.2004.03.022.
18. Livermore DM, Winstanley TG, Shannon KP. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. J Antimicrob Chemother 2001;48:87-102. doi: 10.1093/jac/48.suppl_1.87.
19. Baker C, Pradhan A, Pakstis L, Pochan DJ, Shah SI. Synthesis and antibacterial properties of silver nanoparticles. J Nanosci Nanotechnol 2005;5:244–9. DOI: 10.1166/jnn.2005.034.
20. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli. Appl Environ Microbiol 2007;73:1712-20. doi: 10.1128/AEM.02218-06.
21. Liu X, Ma L, Chen F, Liu J, Yang H, Lu Z. Synergistic antibacterial mechanism of Bi2Te3 nanoparticles combined with the ineffective β-lactam antibiotic cefotaxime against methicillin-resistant Staphylococcus aureus. J Inorg Biochem 2019;196:110687. doi: 10.1016/j.jinorgbio.2019.04.001.
22. Kang F, Alvarez PJ, Zhu D. Microbial extracellular polymeric substances reduce Ag+ to silver nanoparticles and antagonize bactericidal activity. Environ Sci Technol 2014;48:316-22. doi: 10.1021/es403796x.
23. Allahverdiyev AM, Kon KV, Abamor ES, Bagirova M, Rafailovich M. Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. Expert Rev Anti Infect Ther 2011;9:1035-52. doi: 10.1586/eri.11.121.