Combination Therapy for MRSA Super Bugs: Biofilm Targeted Drug Loaded Nanoparticles as a New Approach for Difficult-to-Treat Infections

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ABSTRACT

Introduction: Methicillin Resistant *Staphylococcus aureus* (*MRSA*) is a genetically variant strain from other strain of *Staphylococcus aureus*, generally resistant to the βeta-lactams antibiotics that include methicillin and other drugs in this class, emergence of drug-resistant S. aureus strains poses a serious threat.

Objectives: In the current study, a novel drug delivery system was established by chemically synthesized Silver Nanoparticles (AgNPs), further incorporated by known potential anti-MRSA drug (Vancomycin) to enhance its efficacy and potentially reduce the risk of resistance.

Methodology: For this purpose, AgNPs were synthesized using chemical reduction method. Specifically, silver nitrate (AgNO3, 169.87 g/mol) dissolved in 100 ml of Deionized water at a 1mM concentration. Separately, the sodium borohydride (NaBH4, 37.83 g/mol) solution was prepared by dissolving the required amount of sodium borohydride in 50 ml distilled water. Furthermore AgNPs were confirmed and characterized using UV-Vis spectrometer, Scanning Electron Microscopy (*SEM*) with Energy Dispersive X-Ray Analysis (EDX) and Fourier transform infrared (FTIR). Antimicrobial activity of AgNPs, silver nanoparticles conjugated vancomycin (AgNPs-VAN) and vancomycin alone against MRSA strain determined using agar well diffusion method. The Minimum Biofilm Inhibitory Concentration (MBIC) evaluated using Microtiter Plate Method (MtP). Lastly, the most inhibited strains at phenotypic level have analyzed at gene level to monitor the biofilm associated gene expression using RT-PCR.

Results: The AgNPs have shown increased antibacterial activity at low molar ratios (1mm) of silver nitrate. The AgNPs showed increased antibacterial activity with an average diameter of 6 mm of the inhibitory zone. The synergetic effect of this combination (AgNPs-VAN) also outstanding. The Zone of inhibition was higher for Silver Nanoparticles Conjugated Drug (AgNPs-VAN) (1 mM, 15mm) than the corresponding drug alone (30 µg: 8 mm) against MRSA. The Zone of inhibition was higher for Silver Nanoparticles Conjugated Drug (AgNPs-VAN) than the corresponding drug alone against MRSA.

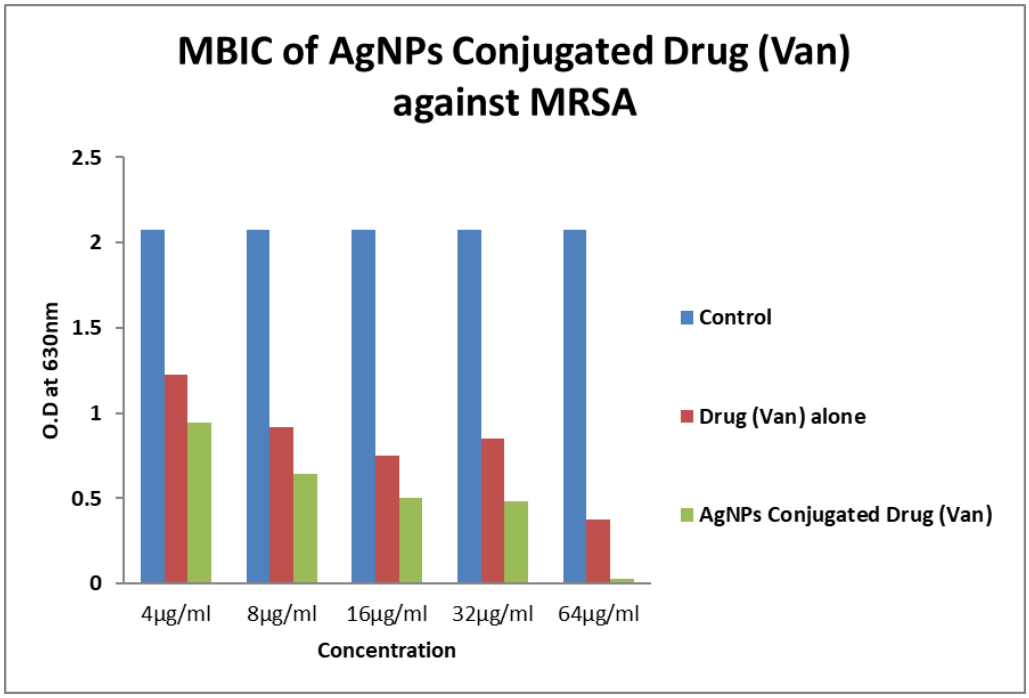
Overall, AgNPs conjugated Drug (AgNPs-VAN) showed 54–98% biofilm inhibition at the concentration of 4-64 µg/mL, 98% is the highest antibiofilm inhibition at the 64 µg/mL concentration and MBIC, 54% was exhibited at the 4 µg/mL concentration. Drug alone results showed lower biofilm inhibition (MBIC: 41% at the 4 µg/mL concentration) as compare AgNPs conjugated drug. Investigation showed, AgNPs conjugated vancomycin had shown to inhibit biofilm, resulting in a reduction of effective dose of vancomycin, ranging from forth-fold to fifth-fold reduction. Furthermore, investigation also showed that the silver nanoparticles conjugated Vancomycin not just reduced the formation of biofilm, but it also reduced biofilm associated gene (icaD and fib) expression of MRSA strains.

Conclusion: Findings suggest that these nanoparticles conjugate (AgNPs-VAN) hold promise as antibacterial and antibiofilm agents for treating multidrug-resistant bacteria, particular MRSA. The combination therapy demonstrated superior efficacy compared to monotherapy, offering potential solutions to combat the challenges posed by MRSA infections.

Keywords: MRSA, Silver Nanoparticles (AgNPs), Antibacterial, Antibiofilm, Synergetic effect

Microorganism	AgNPs (10 mM)	AgNPs (1 mM)	VAN (30 µg)	AgNPs-VAN (10 mM)	AgNPs-VAN (1 mM)
MRSA	3 ± 1	6 ± 1	8 ± 1	15 ± 1	

Antibacterial Activity of AgNPs, VAN, and AgNPs-VAN against MRSA and MSSA



Biofilm Inhibition (%) of AgNPs conjugated Drug (VAN) and drug alone (VAN)

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