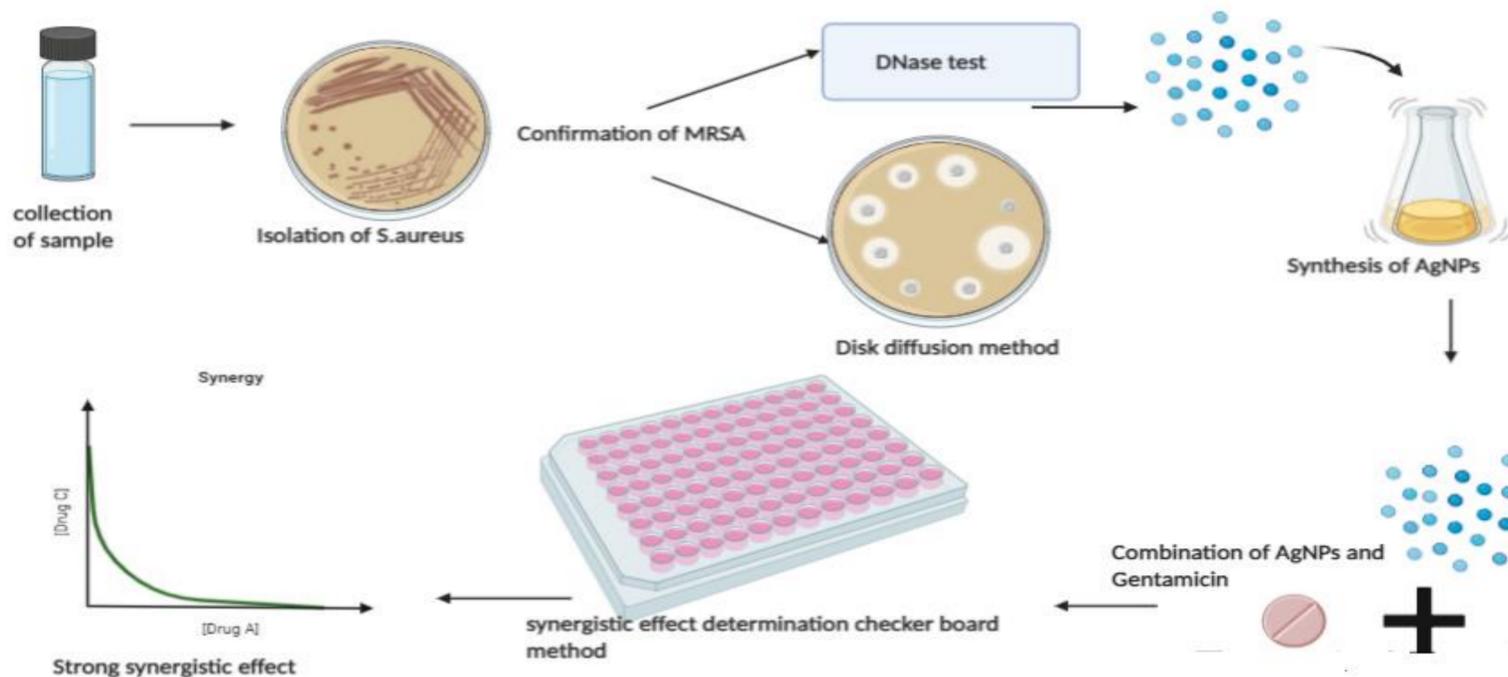


## Antibacterial synergy of Gentamicin with Silver Nanoparticles Against Methicillin-Resistant *Staphylococcus aureus*



## Discussion

The sample Kbrn 13 was taken from the hospital. MRSA strain was confirmed by the biochemical tests. MRSA strain was killed at a very high dose of 12000 µg/ml when gentamicin was applied alone, however, the dose was reduced to 375 µg/ml when applied in combination with silver nanoparticles. According to FIC Index silver nanoparticles and gentamicin combination showed a synergistic effect with FIC index of 0.53125. The synergistic effect was observed with a high rate with no antagonistic activity suggests that the combination of gentamicin with silver nanoparticles can overcome the resistance develop by MRSA strains against gentamicin and to treat the various diseases caused by MRSA.

## Conclusions

The results of the conducted study showed enhanced synergistic activity of AgNPs in the combination with gentamicin against gentamicin resistant strain of MRSA. Although Various mechanism has been proposed to explain their antimicrobial properties further studies are required to explore their exact antimicrobial mechanism, however, the promising results of this study highlight the potential of silver nanoparticles to be used in combinations with other antibiotics against other resistant strains of bacteria.

## Methods and Materials

### Study area

Aminoglycoside (gentamicin) resistant MRSA isolates (already stored at -80°C) were subcultured from glycerol stokes. This study is conducted at the Department of Pharmacy Kohat university of Science and technology

### Synthesis of Silver NPs

Silver NPs are prepared according to our established protocol. This method involves the mixing of silver nitrate solution and gelatin solution on a magnetic stirrer for a specific time period. Briefly, the gelatin solution is prepared in a beaker at 50°C. Subsequently, a different amount of silver nitrate solution is added to the gelatin solution with the help of a micropipette on a magnetic stirrer.

### Characterization of Silver NPs

The Silver NPs were characterized using UV-Visible Spectrometry and Dynamic Light Scattering (DLS).

### Checkerboard assay

For the determination of the combined effects of the drug (gentamicin) and silver nanoparticles on the MRSA, the checkerboard method was used. For this purpose, a 96-well microtiter plate was used. According to the checkerboard method two-fold dilutions of both antibiotics (gentamicin) and silver nanoparticles were at least double to MIC distributed in each tube. The total volume of 1ml was made in each well by distributing 50µl of the inoculum along with drug (gentamicin) and silver nanoparticles dilutions in the same well in the vertical and horizontal directions in 96-well microtiter plate, respectively. After incubation of 24h at 37 °C plates were evaluated and the results obtained were interpreted by fractional inhibitory concentration index (FICI). The effect obtained by the combination of both drugs is considered to be synergistic when the FICI value is ≤0.5 and the effect is antagonistic when it was >4, and no interaction when the FICI value is in the range of 0.5–4.0.

## Results

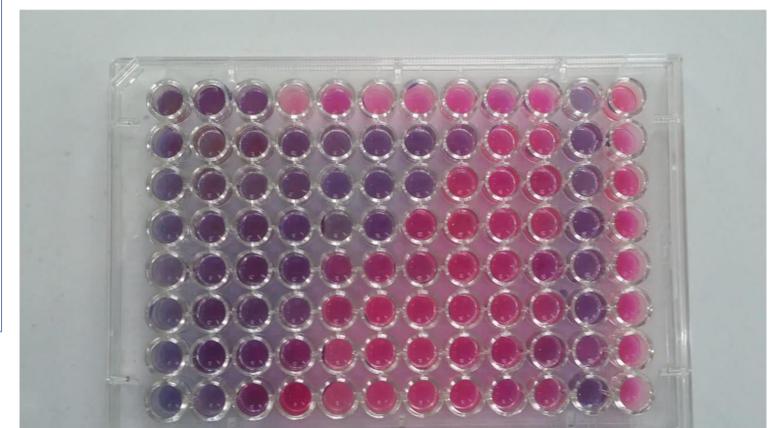
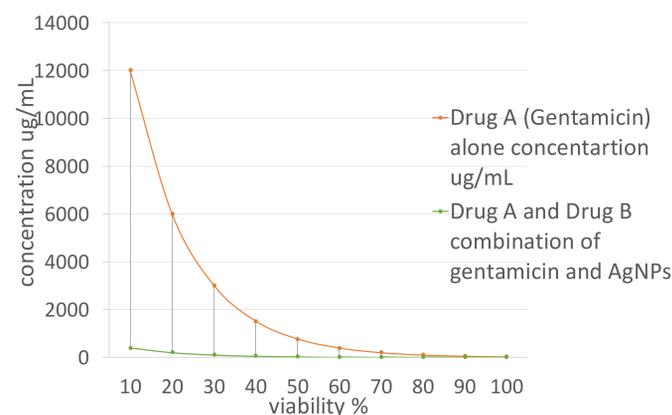
Both drugs (gentamicin and silver nanoparticles) were active against the tested strains of methicillin-resistant staphylococcus aureus (MRSA).

Gentamicin showed a lower MIC that is 375 µg/ml when used in combination with silver nanoparticles which was previously 12000 µg/ml when gentamicin was tested alone against MRSA while silver nanoparticles also showed a lower MIC of 15.625 µg/ml when combined with gentamicin.

The combination of silver nanoparticles and gentamicin resulted in a synergistic effect over the growth inhibition of methicillin-resistant staphylococcus aureus with FIC = 0.53125.

**Results were interpreted using NCCLS guidelines.**

**Figure 1.** Showing the drug A ( gentamicin) alone effect on MRSA which is completely killed at a high dose of 12000 ug/mL while the combination of Gentamicin with silver nanoparticles killed the MRSA at a very low dose of 375 ug/mL showing strong synergistic effect.



**Figure 2:** Synergistic effect determination (Checker board assay)



**Figure 3:** Confirmation of MRSA by Disk Diffusion method

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