

Conjugating azoimidazoles and gold nanoparticles for a synergistic antimicrobial effect against bacteria and yeasts

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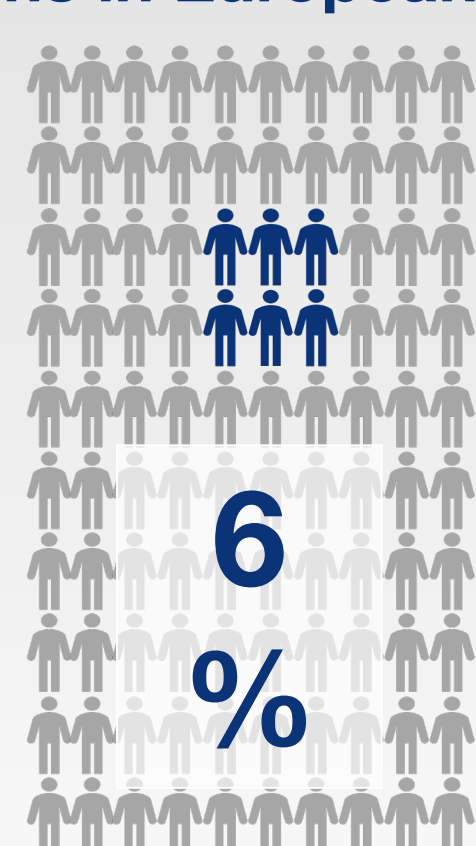
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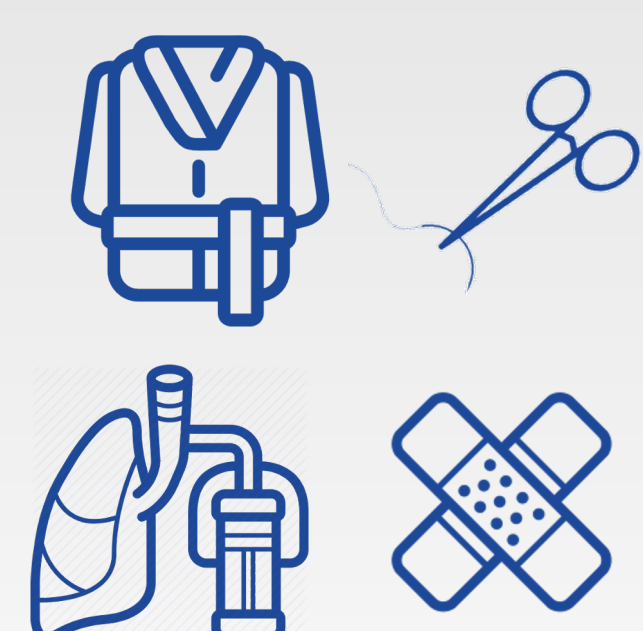
Introduction

Biomedical textiles are frequently related to healthcare-associated infections (HAIs):

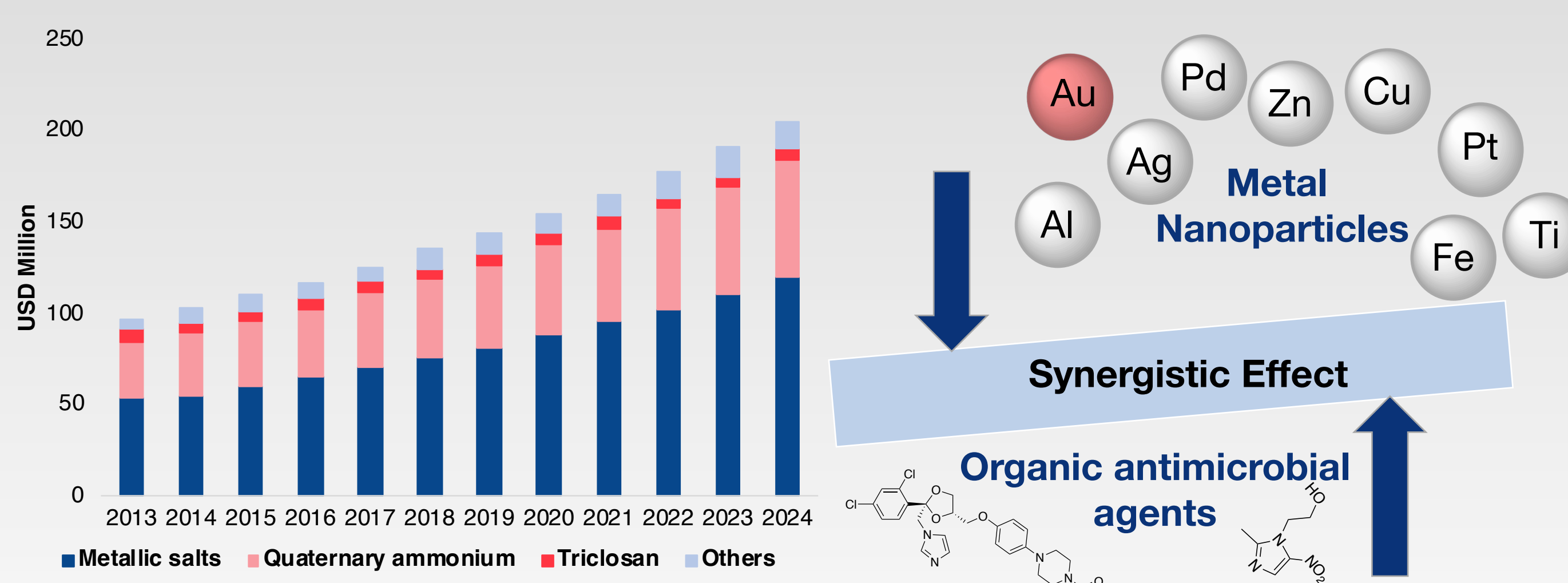
Prevalence of Healthcare-associated Infections in European Hospitals



Biomedical Textiles



Agents acting in synergy can be a powerful tool to fight against pathogens:

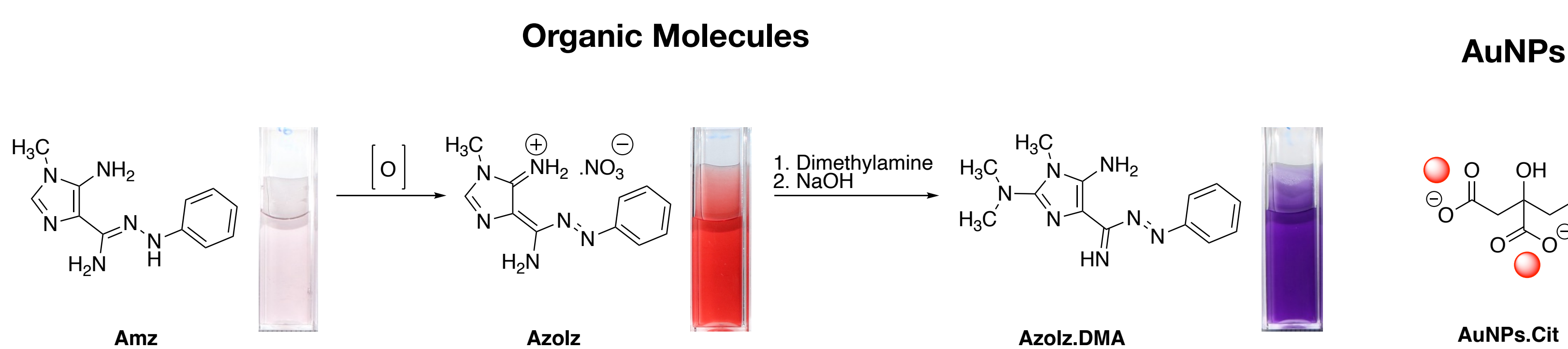


- Increases the concentration of antimicrobial agents in cell membranes;
- Facilitates the transport of antibiotics to the interior of the pathogens' cells;
- Increases the permeability of the membranes;
- Reacts with proteins, inhibits enzymes and deregulates the efflux pump systems.

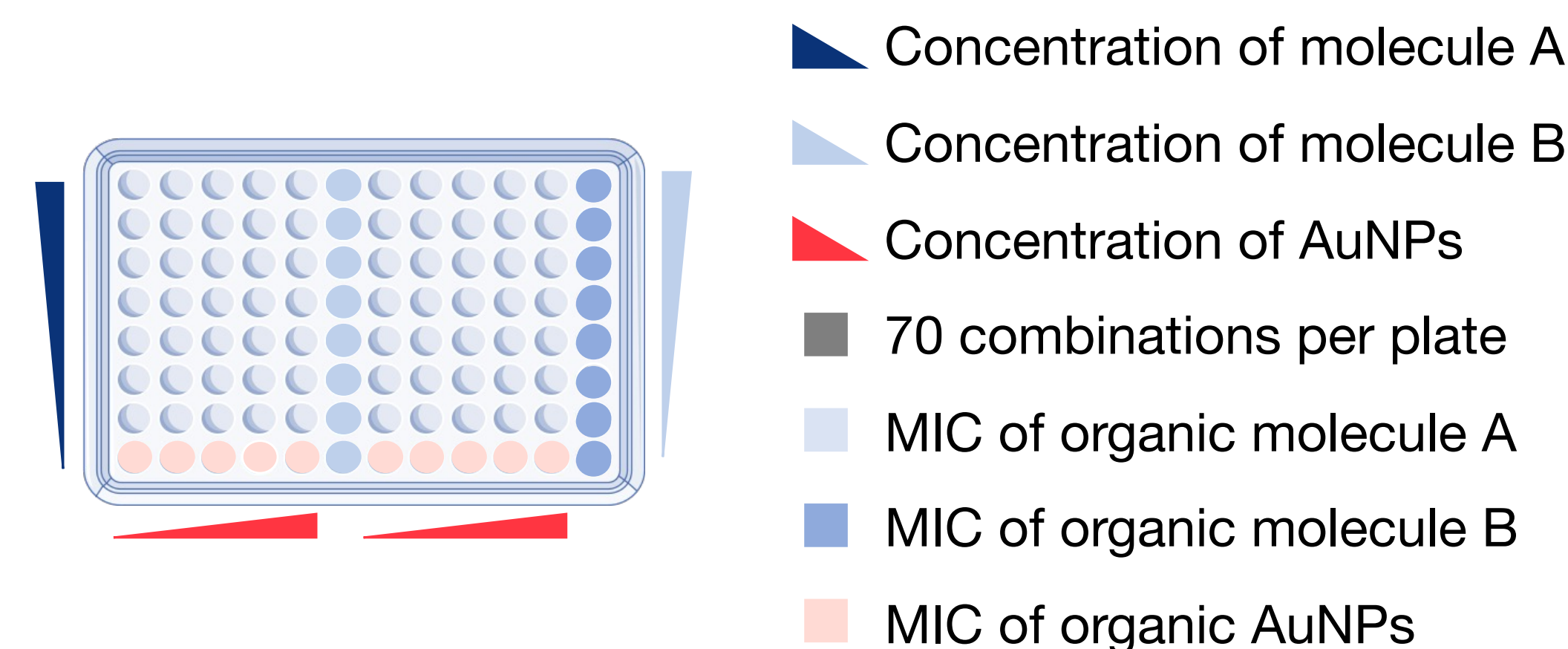
There is a demand for long-lasting, broad-spectrum and more efficient antimicrobial agents to functionalize these materials and avoid cross-contamination.

Methodology

Preparation of organic molecules, AuNPs dispersion and their combination:



Schematic representation of the checkerboard assay:



Data reported in terms of microorganism growth and fractional inhibitory concentration index (FICI):

$$\text{Microorganism growth (\%)} = \frac{\text{OD agent combination well} - \text{OD background}}{\text{OD agent free well} - \text{OD background}}$$

Microorganism growth $\leq 20\%$

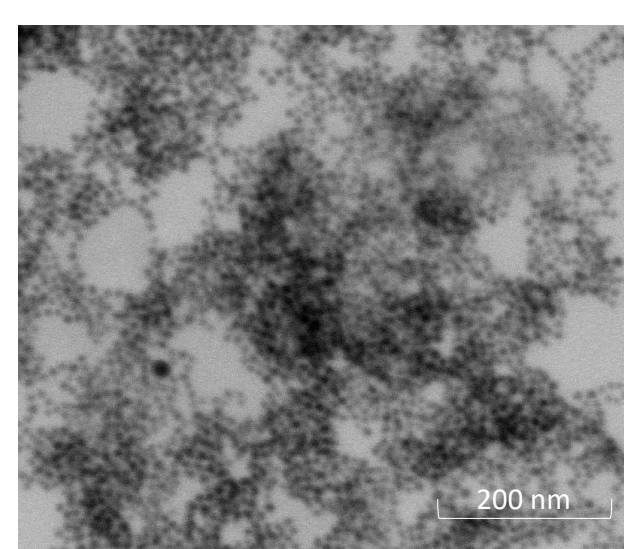
$$\text{FICI} = \frac{\text{MIC of organic molecule in combination}}{\text{MIC of the organic molecule alone}} + \frac{\text{MIC of AuNPs in combination}}{\text{MIC of the AuNPs alone}}$$

FICI ≤ 0.5 , Synergistic
0.5 < FICI ≤ 1.0 , Additive

Results

Morphological analysis (STEM) and dynamic light scattering (DLS) of AuNPs and mixtures with organic molecules:

STEM image of AuNPs



DLS and zeta potential results

	[Organic molecule+AuNPs] ($\mu\text{g} \cdot \text{mL}^{-1}$)	Zeta Potential (mV)	Size (nm)	Polydispersity Index
AuNPs	0+50	-41.2 \pm 1.6	32.1 \pm 1.0	0.2 \pm 0.01
Amz+AuNPs	32+50	-12.6 \pm 0.8	6239.0 \pm 411.5	1
Azolz+AuNPs	32+50	-23.3 \pm 1.6	70.5 \pm 0.2	0.4 \pm 0.02
Azolz.DMA+AuNPs	32+50	-16.6 \pm 0.6	2968 \pm 226.3	1

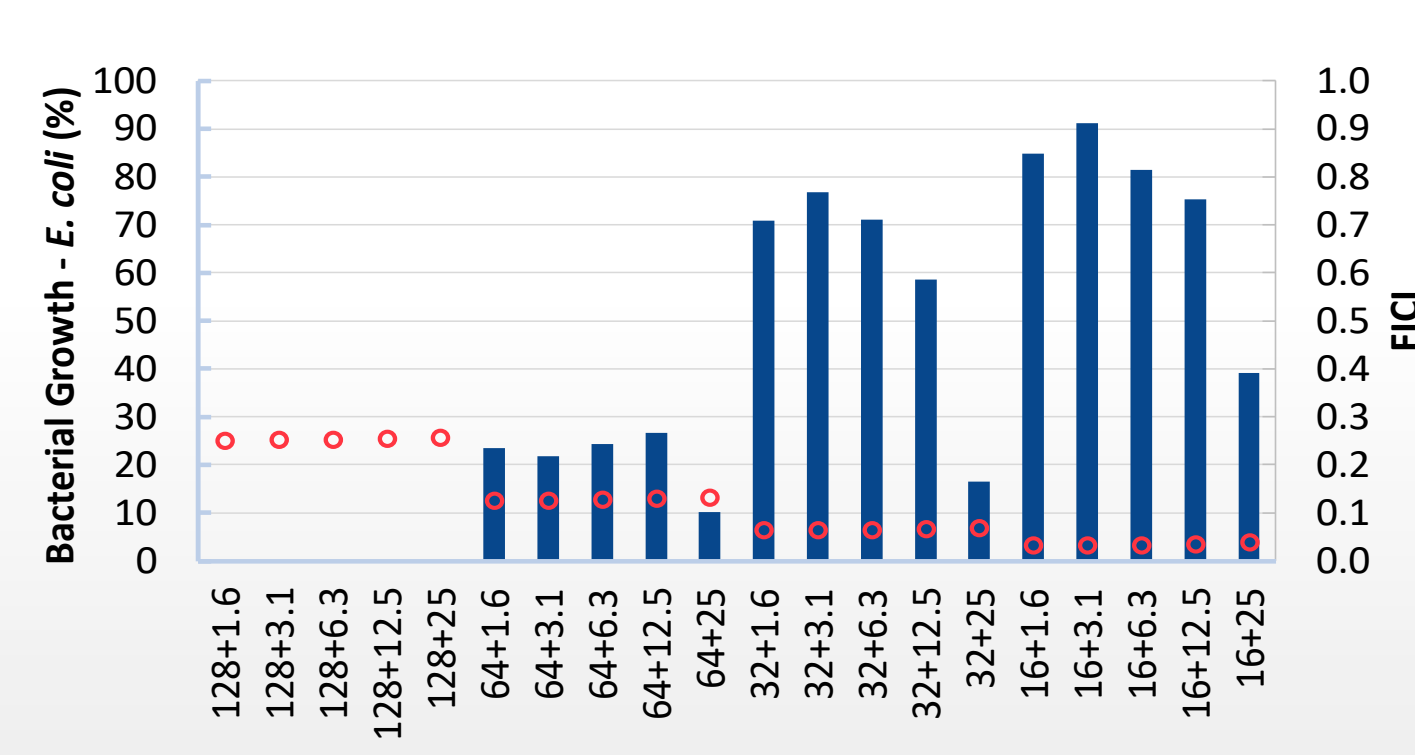
- STEM images of AuNPs showed spherical nanoparticles with a size of 15 \pm 5 nm;
- The size of AuNPs increased when combined with the molecules;
- The zeta potential of the AuNPs decreased when combined with the molecules.

Minimum inhibitory concentration (MIC) and antimicrobial combination assay (checkerboard results):

Minimum inhibitory concentration ($\mu\text{g} \cdot \text{mL}^{-1}$) values that were used for calculating the FICI

	Amz ⁺	Azolz ⁺	Azolz.DMA ⁺	AuNPs
<i>S. aureus</i>	128	128	64	>100
<i>E. coli</i>	128	128	128	>100
<i>C. albicans</i>	8	4	8	>100
<i>C. krusei</i>	16	8	8	>100

Checkerboard test by combining Azolz.DMA with AuNPs, bars represent the microbial growth and dots the FICI



Mixtures of organic molecules (blue) and AuNPs (red) ($\mu\text{g} \cdot \text{mL}^{-1}$) that present a synergistic (S) or additive (A) effect

	Amz ⁺	Azolz ⁺	Azolz.DMA ⁺
<i>S. aureus</i>	32 12.5	S 25	A 32 25
<i>E. coli</i>	-	-	32 25
<i>C. albicans</i>	4 3	A	-
<i>C. krusei</i>	8 12.5	A	-

Conclusion

- The molecules were adsorbed on the AuNPs surface detected by DLS results;
- Antimicrobial synergistic and additive results were found using the checkerboard assay;
- Synergistic concentrations increased the antimicrobial activity using lower concentrations;
- Strategy helpful to fight against HAIs, polymicrobial infections and functionalize materials.

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