Boosting Antibiotic Activity Against *Staphylococcus Aureus* Methicillin Resistant And Susceptible Strains By Photodynamic Inactivation

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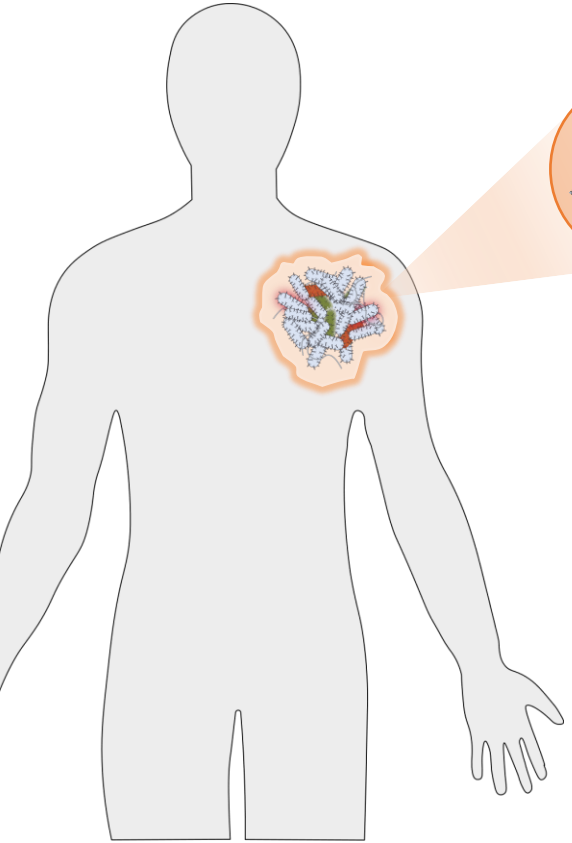
U.PORTO FEUP FACULDADE DE ENGENHARIA UNIVERSIDADE DO PORTO
ALiCE ASSOCIATE LABORATORY IN CHEMICAL ENGINEERING
Lepabe Laboratory for Process Engineering, Environment, Biotechnology and Energy
BEL Biofilm Engineering Lab

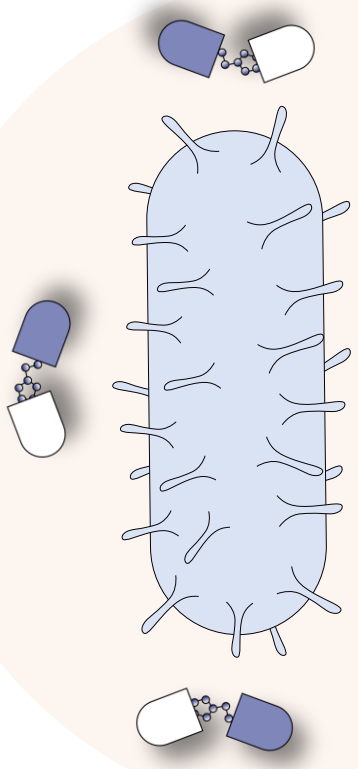
Wound Infections: Persistent Challenge of Antibiotic Resistance



Wound infections are a major challenge for healthcare professionals

Acute wound infections (AWIs)

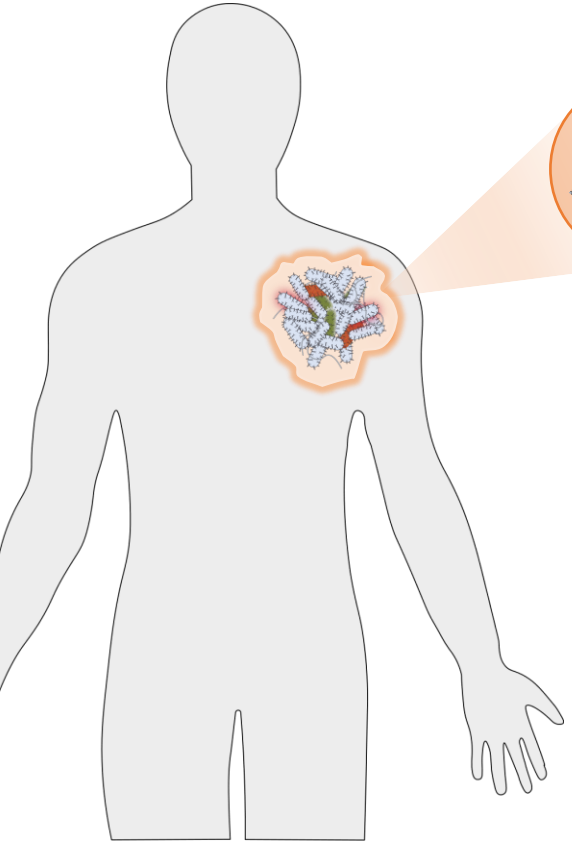
Chronic wound infections (CWIs)

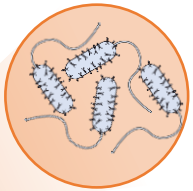


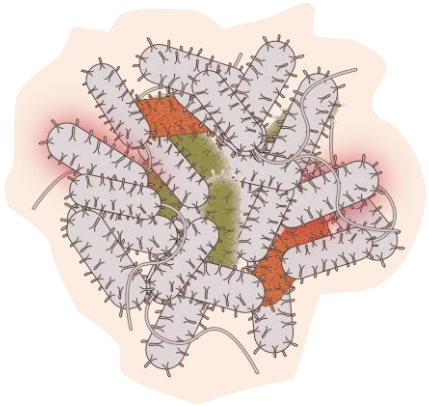
Antibiotic Resistance

Gonçalves, A.S., *The action of phytochemicals in biofilm control.* Natural Product Reports, 2023.

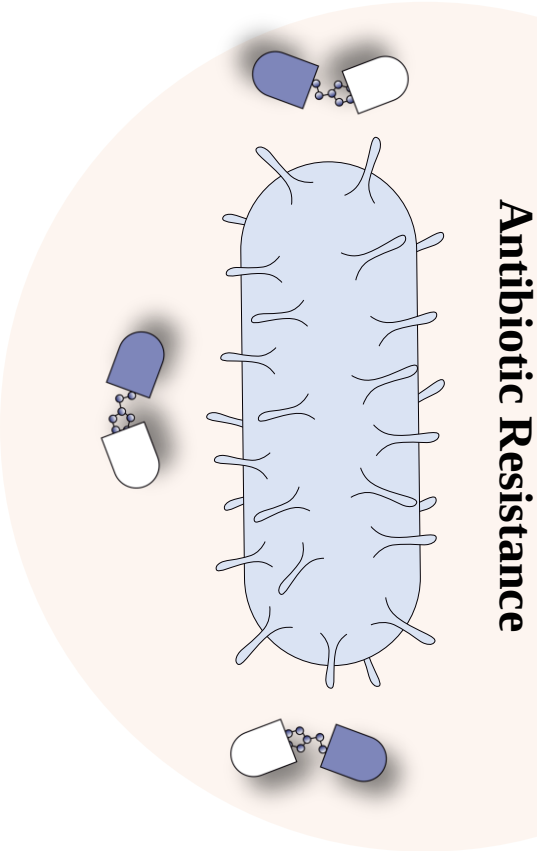
Wound Infections: Persistent Challenge of Antibiotic Resistance







Biofilm formation increases even more such difficulty, preventing the complete healing of CWIs



Antibiotic Resistance

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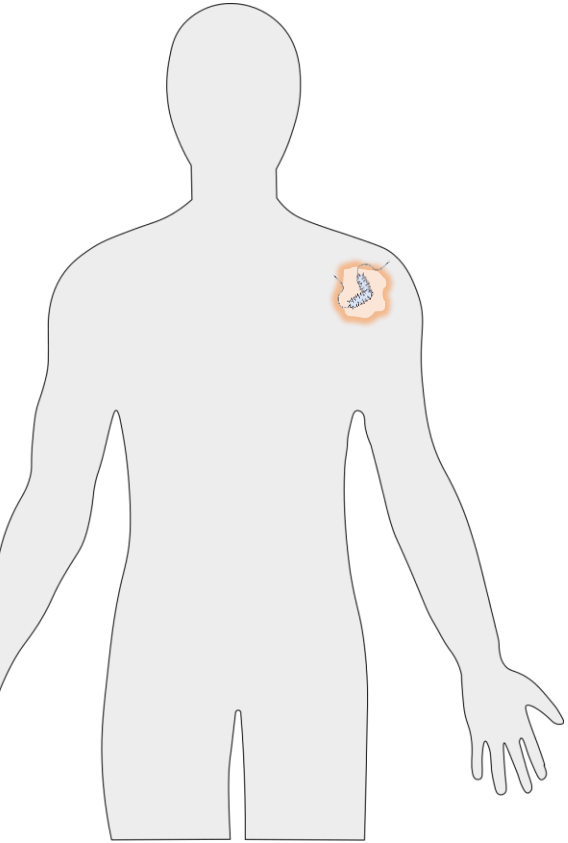
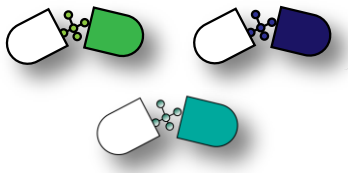
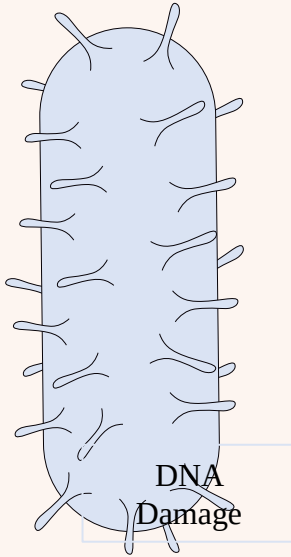
Breaking Resistance's Barrier: Antibiotic/Phytochemical Combinations





The only available strategy to combat bacterial wound infections



Antibiotic Resistance

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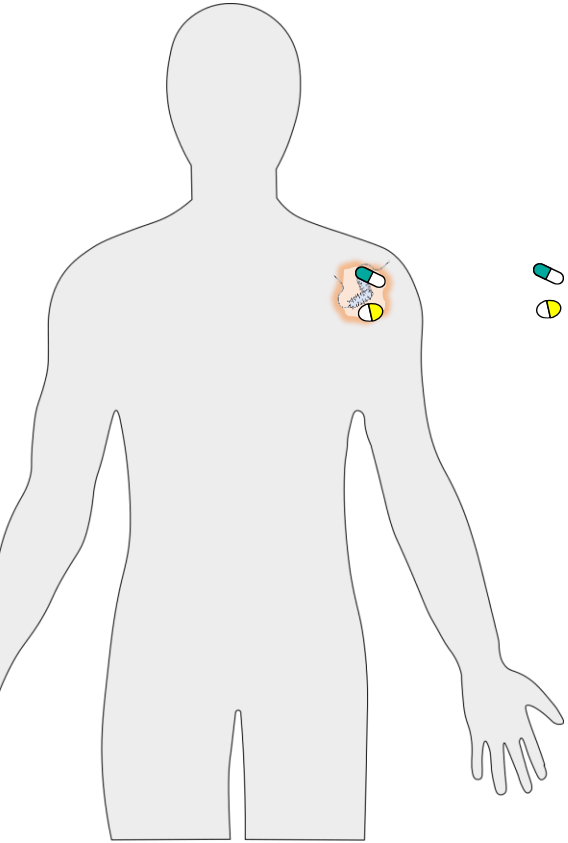
Breaking Resistance's Barrier: Antibiotic/Phytochemical Combinations



The only available strategy to combat bacterial wound infections

Antibiotic Combinatorial Therapy

Phytochemicals (Antibiotic Adjuvants)

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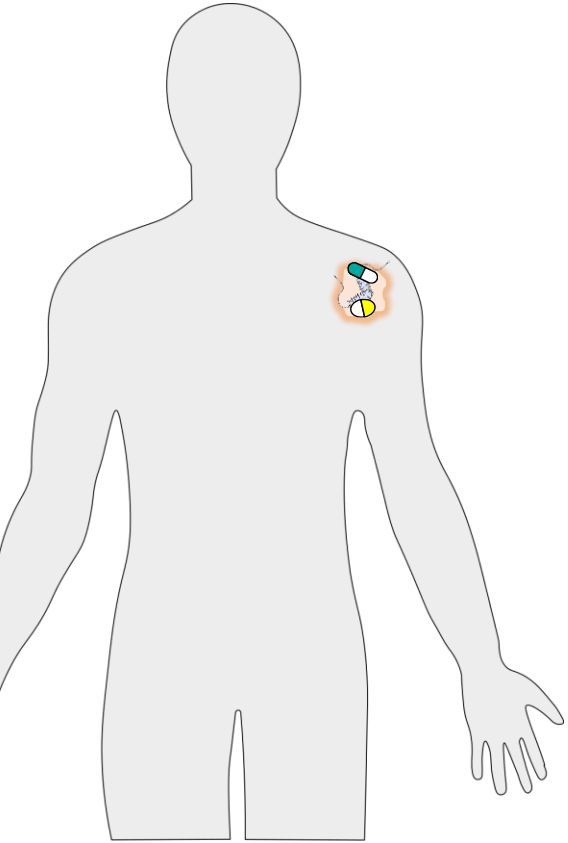
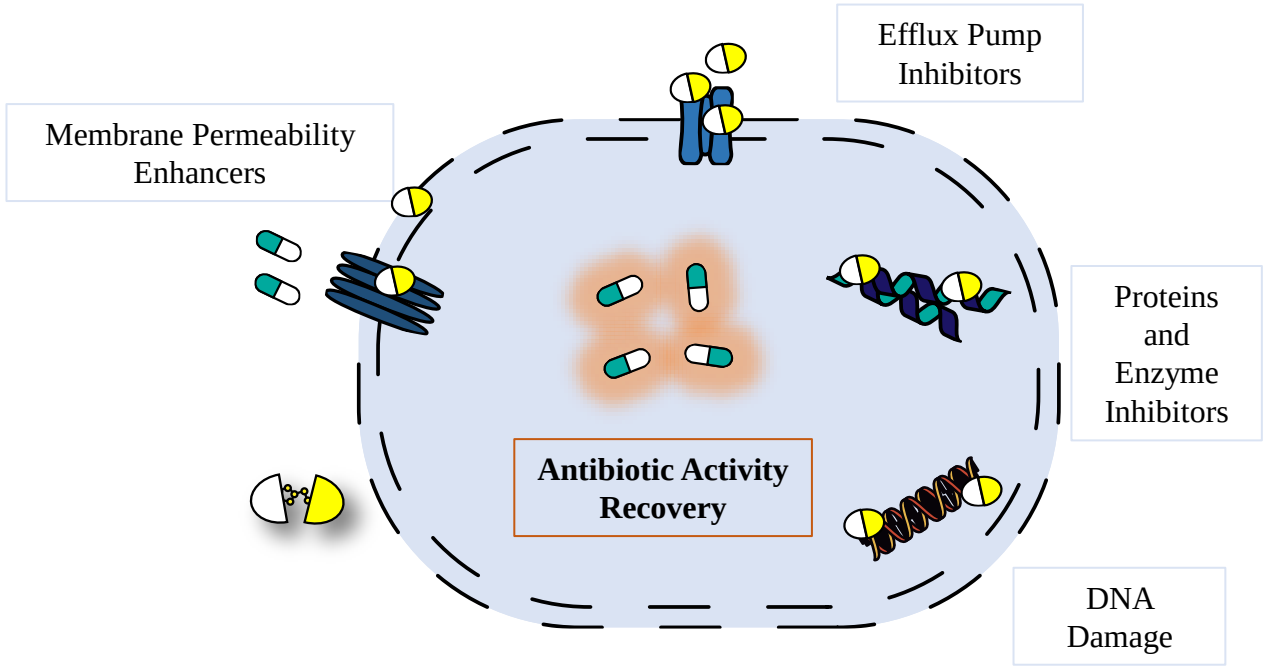
Breaking Resistance's Barrier: Antibiotic/Phytochemical Combinations





Multi-Target Antimicrobial Activity

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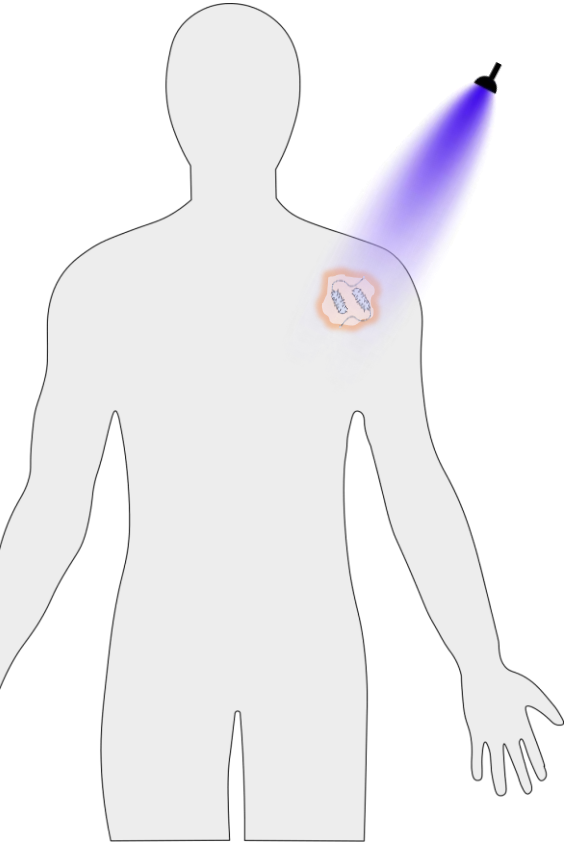
Breaking Resistance's Barrier: Antimicrobial Photodynamic Inactivation



Antimicrobial Photodynamic Inactivation (aPDI) is a promising strategy to treat AWIs.

Localized and non-selective effect, reducing the ability to acquire resistance.

Requires the presence of oxygen, a non-toxic photosensitizer (*e.g.* some phytochemicals) and light.

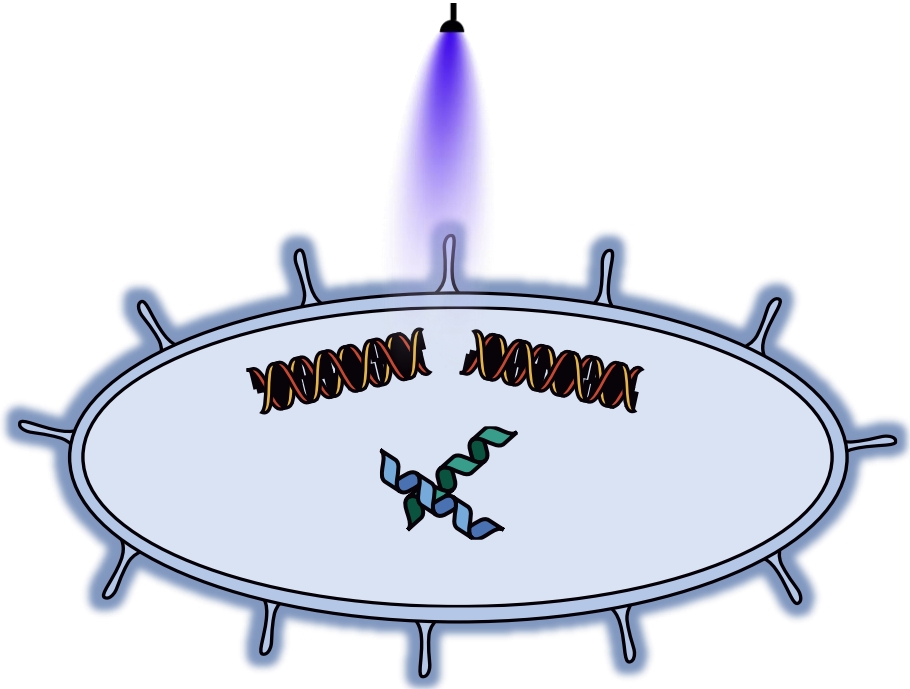
Gonçalves, A.S., *The action of phytochemicals in biofilm control.* Natural Product Reports, 2023.

Breaking Resistance's Barrier: Antimicrobial Photodynamic Inactivation

Induction of cell cytotoxicity through the production of reactive oxygen species (ROS).

Transition from a low energy level (ground state) to a high energy level (excited state).

Irreversible damage to cellular structures, which can lead to cell death.



DNA | Proteins | Membrane

Gonçalves, A.S., *The action of phytochemicals in biofilm control.* Natural Product Reports, 2023.

Aims

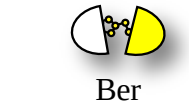
Evaluate, for the first time, the synergy between phytochemical-antibiotic combinations and photodynamic activation to overcome antibiotic resistance and, consequently, increase the success of inactivating *Staphylococcus aureus* AWIs.

Antibiotic-berberine (Ber) combinatorial effect

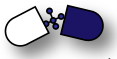
Photodynamic activation of Ber-Antibiotic combinations (BAc) to inactivate *S. aureus* strains

Ber Blue Light Photoactivation

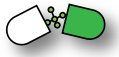
Materials and Methods



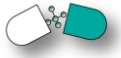
Ber



Mupirocin (Mup)

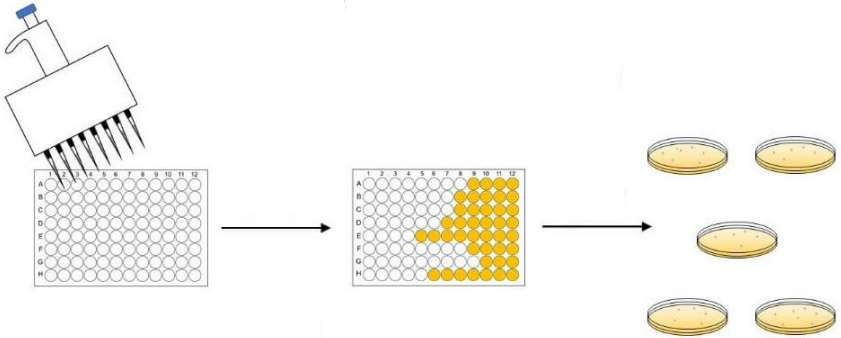


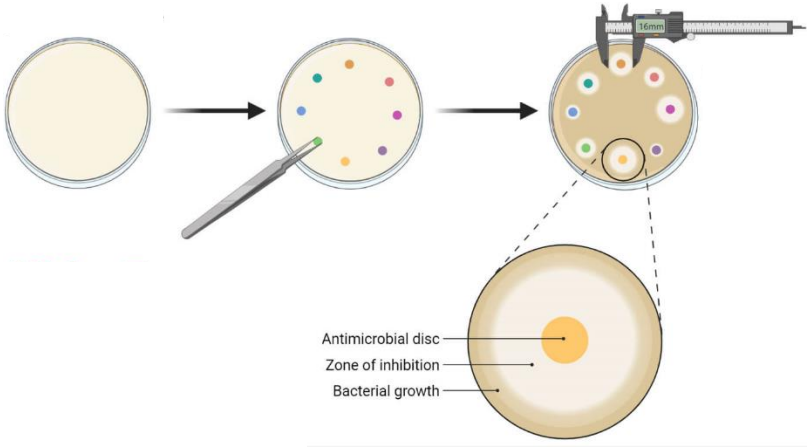
Gentamicin (Gen)



Tobramycin (Tob)

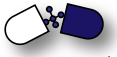
- Minimum Inhibitory Concentration (MIC)
- Minimum Bactericidal Concentration (MBC)
- Disc Diffusion Test (DDT)

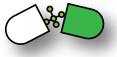




Materials and Methods


Ber


Mupirocin (Mup)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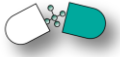
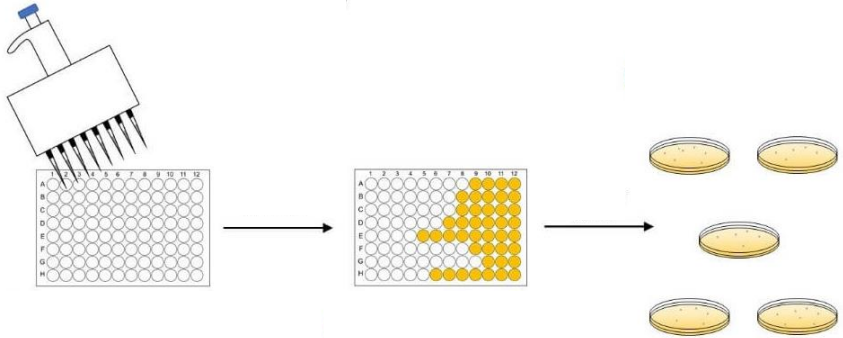
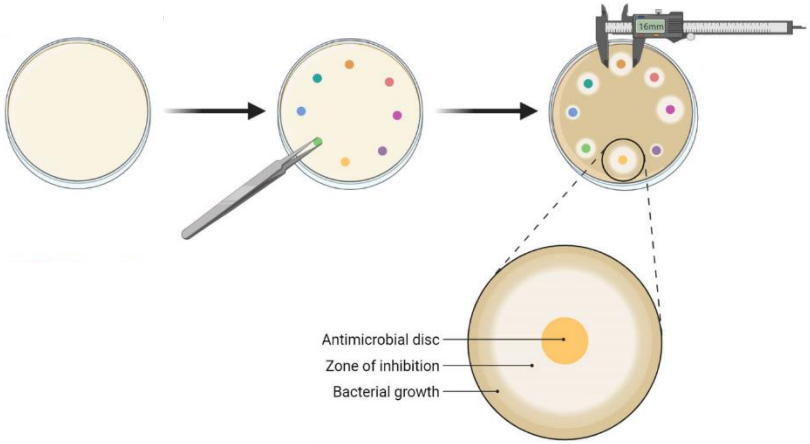

Tobramycin (Tob)

- Minimum Inhibitory Concentration (MIC)
- Minimum Bactericidal Concentration (MBC)
- Disc Diffusion Test (DDT)

S. aureus CECT 976 (methicillin-susceptible-MSSA)

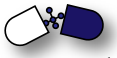
S. aureus MJMC568-B (methicillin-resistant-MRSA)

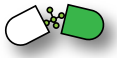




Materials and Methods


Ber


Mupirocin (Mup)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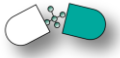
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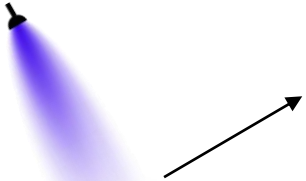
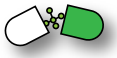

Ber

LED parameters optimization:
420 nm, 30 mW/cm^2, during 5, 10, and 15 min (9, 18, and 27 J/cm^2)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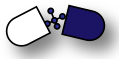
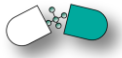
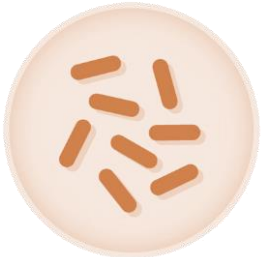

Mup


Tob

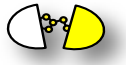
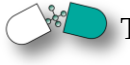
6 h

Culturability assessment (colony forming units per milliliter (CFU/mL))



Results and Discussion – MIC, MBC, and DDT

Table 1. MIC, MBC (µg/mL) Ber and selected antibiotics, as well as the classification for the combinatorial application (CA) of different BAc MSSA and MRSA strains.

Compound	MSSA			MRSA		
	MIC	MBC	CA Classification	MIC	MBC	CA Classification
 Ber	100	1000	-	800	800	-
 Gen	1	2	Indifferent	>1024	>1024	Potentiation
 Mup	8	8	Potentiation	16	512	Additive
 Tob	4	4	Additive	64	256	Potentiation

Potentiation (+++): $(IZD_{a+p} – IZD_a) \geq 6$ mm; Additive (++): 6 mm > $(IZD_{a+p} – IZD_a) \geq 4$ mm; Indifferent (+): 4 mm > $(IZD_{a+p} – IZD_a)$ > -6 mm; Negative (-): $(IZD_{a+p} – IZD_a) \leq$ - 6 mm.

The MIC and MBC values for MRSA were higher than those determined for MSSA.

Results and Discussion – MIC, MBC, and DDT

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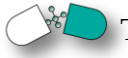 Tob	4	4	Additive	64	256	Potentiation

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Ber-Mup for MSSA, Ber-Gen and Ber-Tob for MRSA with synergistic effect.

Results and Discussion - Ber Antimicrobial Photodynamic Activity

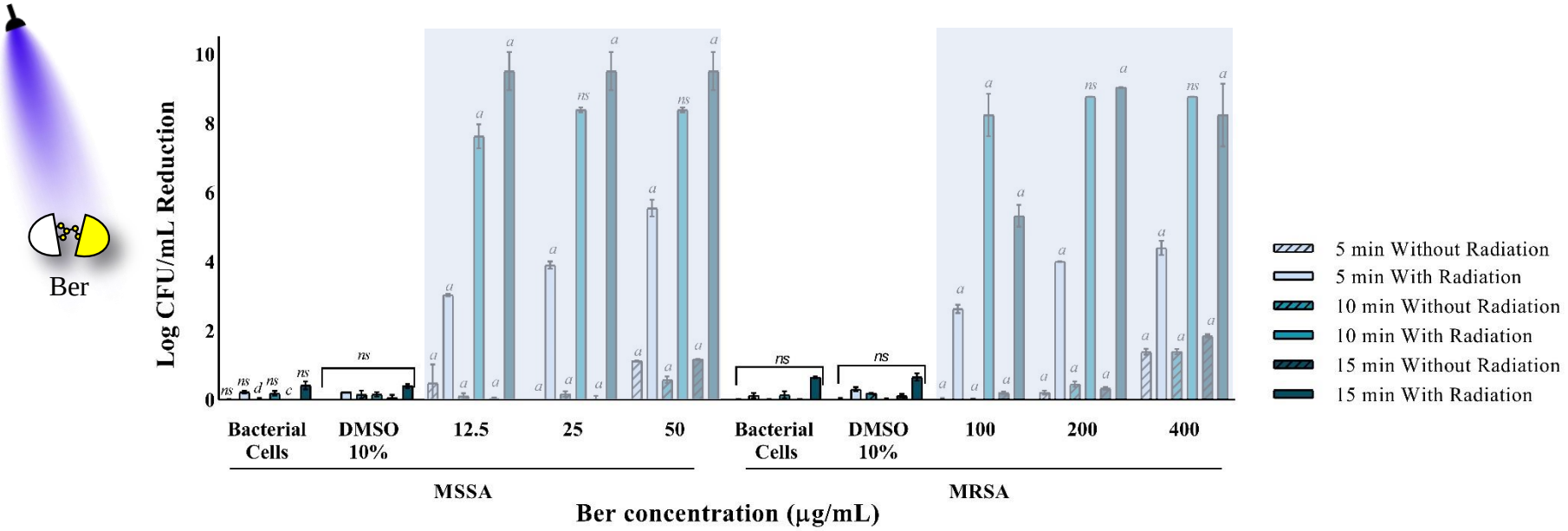
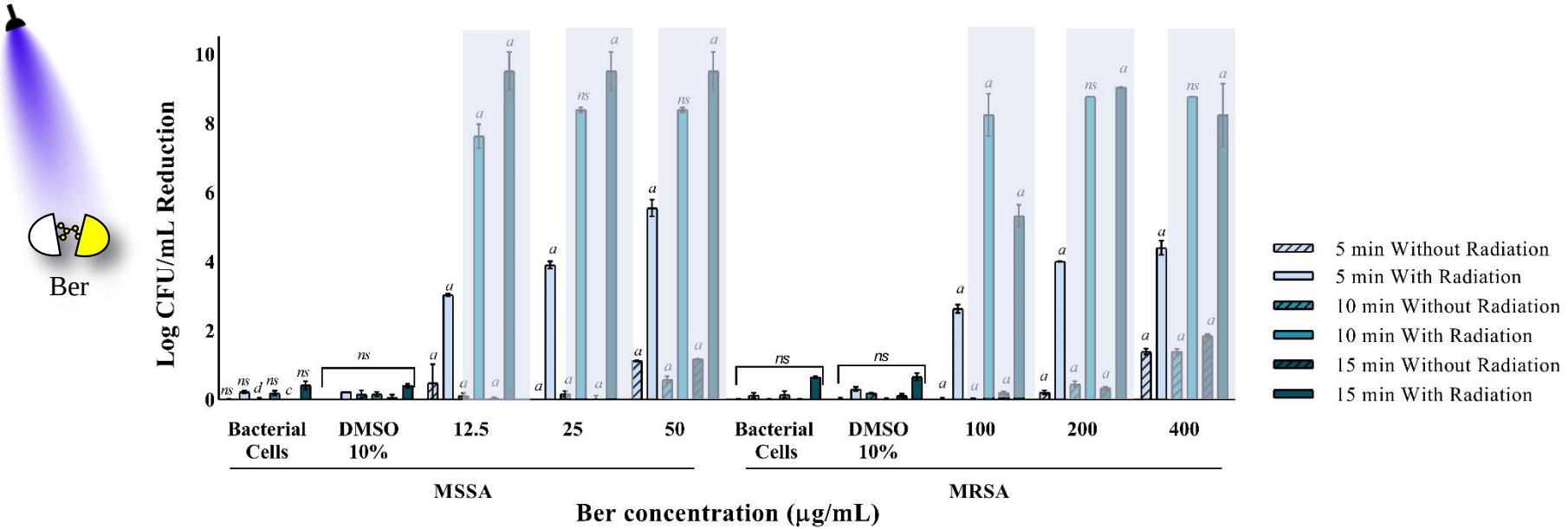


Figure 1. Antimicrobial photodynamic activity of Ber against MSSA and MRSA in terms of culturability (CFU/mL) after 5, 10 and 15 min (9, 18 and 27 J/cm^2) blue light irradiation (420 nm, 30 mW/cm^2). Bacterial cells were incubated in the dark at 37 °C and 150 rpm for 30 min prior to irradiation. No irradiation was applied to the control groups. Data are presented as mean ± standard derivations (SDs)., $P < 0.05$ is given for two independent experiments with at least three replicates. *a* stands for ****; *b* stands for ***; *c* stands for **; *d* stands for *; *ns* stands for non-statistical difference. Statistical analyses were performed between irradiated and non-irradiated bacterial cells and between 5, 10 and 15 min of irradiation.

The photodynamic activation of Ber resulted in an increase of its antimicrobial activity, decreasing its MBC in 80 and 8 times for MSSA and MRSA strains, respectively.

Results and Discussion - Ber Antimicrobial Photodynamic Activity



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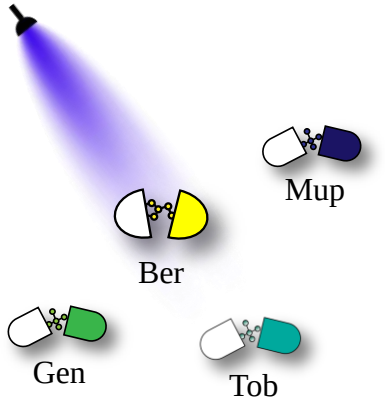
The irradiation time of 10 min was chosen because no significant difference was observed with the application of 27 J/cm^2 compared to 18 J/cm^2. In addition, higher doses of light are associated with toxicity to human cells, therefore lower irradiation times are preferred.

Results and Discussion – BAc Antimicrobial Photodynamic Activation



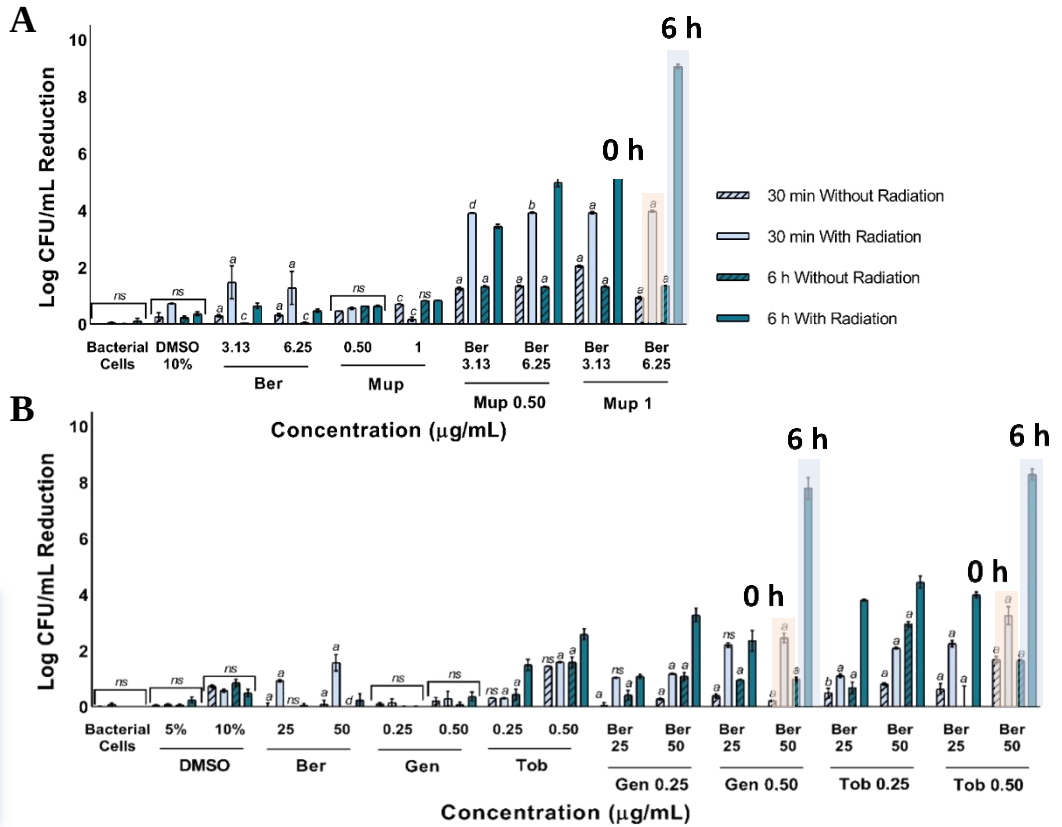
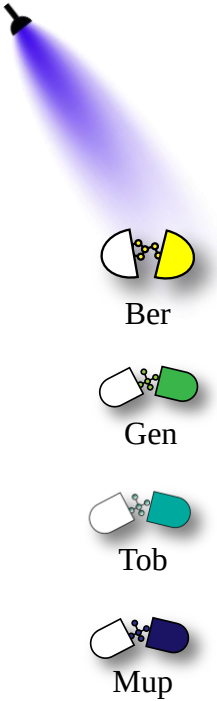


Figure 2. Antimicrobial photodynamic activation of BAc against MSSA (A) and MRSA (B) in terms of culturability (CFU/mL) after 10 min of blue light irradiation (420 nm, 30 mW/cm², 18 J/cm²). Data are presented as mean ± SDs, $P < 0.05$ is given for two independent experiments with at least three replicates. a stands for ****; b stands for ***; c stands for **; d stands for *; ns stands for non-statistical difference. Statistical analyses were performed between irradiated and non-irradiated bacterial cells and between between 30 min and 6 h of incubation..

- All photoactivated BAc inactivated culturability completely after 6 h incubation.
- 6 h incubation has shown a key role in increasing the antibiotic activity.

Concluding Remarks



Ber has potent photodynamic capabilities against MSSA and MRSA strains.

Photoactivated Ber ability to restore the antibacterial activity of less effective antibiotics.

The results demonstrate the great potential of photoactivated BAc to treat efficiently AWIs and possible prevent the development of CWIs.

Acknowledgments

- This work was supported by: LA/P/0045/2020 (ALiCE) and UIDP/00511/2020 (LEPABE) funded by national funds through FCT/MCTES (PIDDAC);
- Ariana thanks the FCT, for the financial support of her PhD fellowship (2022.10913.BD);
- Anabela Borges thanks FCT, for the Scientific Employment Stimulus—Individual Call—[CEECIND/00823/2021];
- Biofilm engineering Lab (BEL);
- University of Trás-os-Montes e Alto Douro (UTAD);
- Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA).

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Cristiana Pereira
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Acknowledgments

Thank you for your attention!

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