Antibiotic susceptibility of *Staphylococcus aureus* biofilms in prosthetic joint infections: insights for optimizing treatment strategies

L.T.D. Speijker[A,B], J. Dingemans[A,C], J.J. Arts[B,D], P.H.M. Savelkoul[A,B], I.H.M. van Loo[A,B]

A Department of Medical Microbiology, Infectious Diseases and Infection Prevention, Maastricht University Medical Centre, Maastricht, The Netherlands
B Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, The Netherlands
C Laboratory clinical biology and Center of Molecular Diagnostics, Jessa Ziekenhuis Vzw, Hasselt, Belgium
D Department of Orthopaedic Surgery, Maastricht University Medical Centre, Maastricht, The Netherlands

Background

Prosthetic joint infections (PJI) (figure 1)

- Average incidence of PJI between 0.5-2.0% after surgery[1], accounts for 13-31% of revision surgeries[2]
- Difficult to completely eradicate bacteria due to biofilm formation[3]
- Susceptibility testing performed on planktonic cells[4,5]
  - However, biofilm specific eradication concentration may give more reliable results

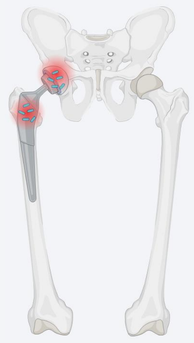

Figure 1: Orthopedic implant infection (BioRender).

Study objectives

- Assess antibiotic susceptibility testing of planktonic and biofilm *Staphylococcus aureus (S. aureus)*
  - Minumum Inhibitory Concentration (MIC), golden standard
  - Minimum Biofilm Eradication Concentration (MBEC)
- Evaluate effectivity of novel antibiotic therapies: fosfomycin and combination therapy with minocyclin

Methods

- MIC – ISO-20776-1[4]
- Biofilm formation on plastic lid with 96 pegs (MBEC assay[6])
- MBEC definition: 99.9% reduction

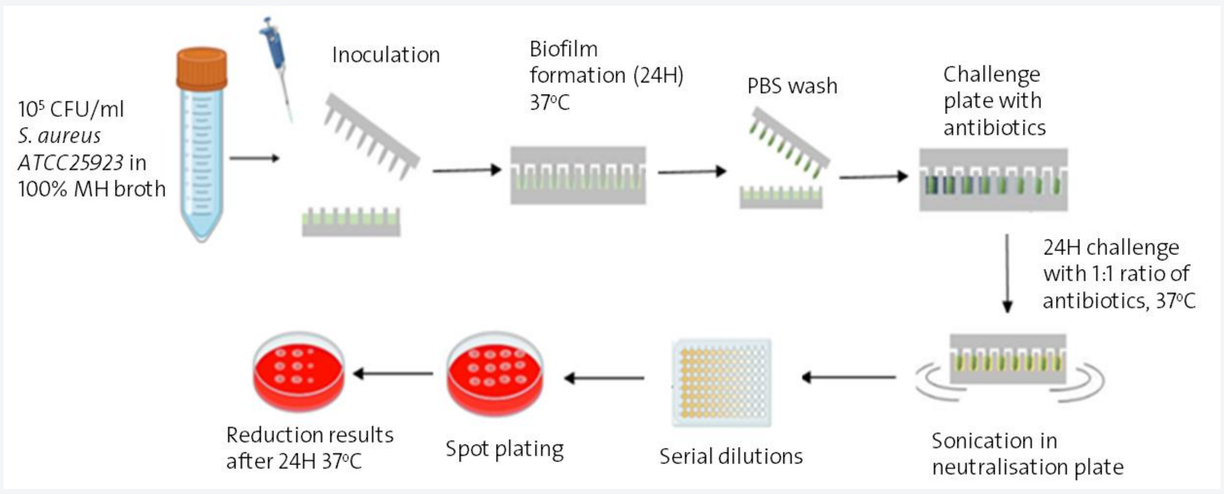

Figure 2: Workflow overview of the MBEC assay (BioRender)

Results

- MBEC up to 32,000 times higher than the MIC (mean±SEM: 6248±2284)
- Fosfomycin good alternative therapy
- Rifampicin combinations consistently more effective against biofilm cells

Table 1: antibiotic concentrations of frequently used antibiotics to treat PJI

	MIC (mg/L)		MBEC (mg/L)	
Antibiotic	+ rifampicin	+ minocyclin	+ rifampicin	+ minocyclin
Flucloxacillin	0.016	0.125	512	512
Vancomycin	0.016	0.25	256	512
Clindamycin	0.016	0.06	0.125	512
Cotrimoxazole	0.016	0.25	0.016	>512
Levofloxacin	0.016	0.125	8	512
Linezolid	0.016	0.125	128	256
Fosfomycin	0.016	0.125	64*	512

*64 mg/L as MBEC including small colony variants (SCVs), without the MBEC would be 0.03 mg/L

Conclusions

Antibiotic concentration amount: MBEC >MIC

Rifampicin combinations demonstrate superior efficacy compared to minocycline combinations

Fosfomycin in combination with rifampicin → effective antibiotic therapy in biofilm infections excluding SCVs

Golden standard (MIC) testing might need to be re-evaluated in patients with biofilm-associated infecitons

Future work

- Further testing of antibiotic resistance in biofilm related PJI-infections
  - Addition of clinical *S. aureus* strains
- Use dynamic model (bioreactor) to more closely mimic *in vivo* environment
- Use scanning electron microscopy imaging of biofilms at the MBEC concentration to visually confirm reduction in bacterial growth

References

[1] Weinstein, E. J., et al., JAMA network open (2023). https://doi.org/10.1001/jamanetworkopen.2023.40457; [2] Perfetti, D. C. et al., The Journal of arthroplasty (2017). https://doi.org/10.1016/j.arth.2017.02.027 ;[3]Ezzariga, N., et al., Cureus (2025). https://doi.org/10.7759/cureus.78673; [5] ISO-20776-1 (2020); [6] EUCAST, https://www.eucast.org/bacteria/methodology-and-instructions/mic-determination/; [6] MBEC Assay® Kit, https://innovotech.ca/biofilm-products/mbec-assay-kit/

Correspondence to: Lydia Speijker, Medical Microbiology, Infectious Diseases and Infection Prevention

lydia.speijker@mumc.nl | lydia.speijker@maastrichtuniversity.nl
https://www.mumc.nl/

Maastricht University
P.O. Box 616
6200 MD Maastricht,
The Netherlands

Maastricht UMC+
P.O. Box 5800
6202 AZ Maastricht,
The Netherlands

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