

Biofilm properties in relation to treatment outcome in patients with first-time periprosthetic hip or knee joint infection

Svensson Malchau Karin^{1,2,*}, Tillander Jonatan^{3,2}, Zaborowska Magdalena^{4,2}, Hoffman Maria^{4,2}, Lasa Iñigo⁵, Thomsen Peter⁴, Malchau Henrik⁶, Rolfson Ola¹, Trobos Margarita^{4,2,*}

¹Department of Orthopaedics, Institute of Clinical Sciences, The Sahlgrenska Academy, University of Gothenburg, Sweden

²Center for Antibiotic Resistance Research (CARE) at University of Gothenburg, Gothenburg, Sweden

³Department of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Sweden

⁴Department of Biomaterials, Institute of Clinical Sciences, The Sahlgrenska Academy, University of Gothenburg, Sweden

⁵ Microbial Pathogenesis Research Unit, Public University of Navarre, Spain

⁶Harvard Medical School, Harvard University, Boston, Massachusetts, USA

***Corresponding authors:**

Margarita Trobos, margarita.trobos@biomaterials.gu.se

Karin Svensson Malchau, karin.am.svensson@vgregion.se

Supplementary Table S1. Biofilm production categories as measured by CFU/peg and the mean crystal violet OD. Biofilm production was further divided in subcategories in order to investigate if there were differences between different biofilm degrees. The breakpoints for this new classification were: non-producer ($OD_{570} < 0.120$), weak ($0.120 < OD_{570} < 0.240$), moderate ($0.240 < OD_{570} < 0.480$) and strong ($OD_{570} > 0.480$).

Biofilm category	CFU/peg	Crystal violet OD
Non	1.9×10^5	0.318
Weak	4.7×10^4	0.603
Moderate	9.3×10^4	0.813
Strong	1.6×10^5	0.973

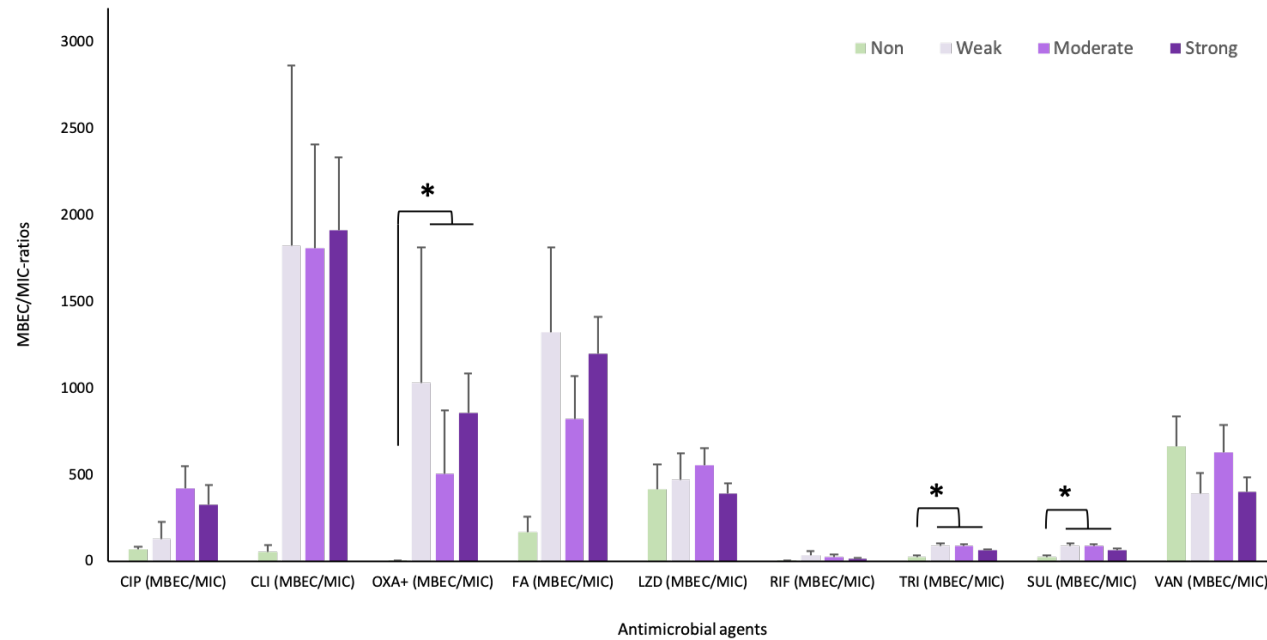
Supplementary Table S2. Inflammatory markers and duration of symptoms in regards to biofilm production. Factors are presented as mean with standard deviation (SD), median with interquartile range (IQR) and the minimum and maximum value. Unit used is mg/L.

Abbreviations: erythrocyte sedimentation rate (ESR), white blood cell count (WBC), C-reactive protein (CRP).

Inflammatory markers	Non-weak biofilm producers	Strong biofilm producers
ESR		
Mean (<i>SD</i>)	55 (26)	47 (31)
Median (<i>IQR</i>)	41 (40-75)	39 (20-83)
Minimum-maximum	20-106	7-120
WBC		
Mean (<i>SD</i>)	7.5 (1.7)	8.4 (3.4)
Median (<i>IQR</i>)	7.5 (6.0-8.7)	7.8 (6.0-9.8)
Minimum-maximum	5.7-11.2	3.6-23.7
CRP		
Mean (<i>SD</i>)	51 (49)	105 (116)
Median (<i>IQR</i>)	43 (17-46)	47 (18-160)
Minimum-maximum	3-170	2-450
Time		
Index surgery to symptom onset		
Mean (SD)	529 (1041)	426 (835)
Median (IQR)	195 (18-405)	107 (20-405)
Minimum-maximum	0-4200	6-4343
Symptom onset to reoperation		
Mean (SD)	31 (29)	50 (110)
Median (IQR)	16 (9-68)	8 (3-58)
Minimum-maximum	1-82	1-452

Supplementary Table S3. Recurrent infections. Characteristics of strains causing confirmed relapses and reinfections in patients with saved strains from subsequent operations.

Patient	Reoperation Number due to PJI (days from prior reoperation)	Procedure	Recurrent PJI (relapse or reinfection)	Strain	Species	MLST	OD	Biofilm	Phenotypic resistance according to MIC	AB received (iv/po)	AB duration (weeks)	Infection resolution
H15	1	stage 1	First-time	6931	<i>S. epidermidis</i>	ST-59	0.224	W	OXA, TRI, SUL	VAN/RIF+CLI	13	
	2 (150 days)	stage 2	Reinfection	6932	<i>S. hominis</i>	-	0.532	S	CLI, OXA, TRI, SUL	VAN/0	1	Yes
H28	1	Stem ex	First-time	6942	<i>S. aureus</i>	ST-25	1.621	S	0	VAN/0	2	No
	2 (12 days)	DAIR, NE	Reinfection	6943	<i>S. aureus</i>	ST-25	0.552	S	CLI, OXA, FA	VAN/RIF+CLI	13	Yes
H50	Arthroscopy ^a	-	First-time ^a	6953	<i>S. aureus</i>	ST-3457	0.518	S	0	-	-	-
	1 (239 days)	DAIR, E	Reinfection	6954	<i>S. epidermidis</i>	ST-215	0.060	N	CIP, CLI, OXA, FA	VAN/TEI	16	Yes
H53	1	stage 1	First-time	6957	<i>S. lugdunensis</i>	ST-3	2.705	S	CLI	CDX/RIF+CIP	7	
	2 (163 days)	stage 2	Reinfection	6956	<i>S. epidermidis</i>	ST-215	0.247	M	CIP, CLI, OXA, FA, RIF	FLX/FLX	2	No
H56	1	DAIR, E	First-time	6960	<i>S. epidermidis</i>	ST-2	0.584	S	CIP, OXA, TRI, SUL	VAN/RIF+CLI	20	No
	2 (174 days)	Cuprevision	Reinfection	6961	<i>S. capitis</i>	-	0.148	W	CLI	VAN/0	2	No
	3 (11 days)	DAIR, E	Relapse (from 6960)	6962 ^b	<i>S. epidermidis</i>	ST-2	0.583	S	CIP, CLI, OXA, RIF, TRI, SUL	VAN/CIP	29	No
H6	1	DAIR, E	First-time	6922	<i>S. aureus</i>	ST-45	1.767	S	0	VAN/TEI	4.5	No
	2 (34 days)	DAIR, E	Reinfection	6923	<i>S. epidermidis</i>	ST-2	0.512	S	CIP, CLI, OXA, FA, RIF, TRI, SUL	VAN/TEI	life	No
			Relapse (from 6922)	6924	<i>S. aureus</i>	ST-45	1.333	S	0	VAN/TEI	life	No
H86	1	stage 1	First-time	6994	<i>S. epidermidis</i>	ST-48	1.431	S	OXA, FA	VAN/RIF+CIP	19	No
				6995	<i>S. epidermidis</i>	ST-48	3.333	S	OXA, FA	VAN/RIF+CIP	19	No
				6996	<i>S. epidermidis</i>	ST-48	3.333	S	OXA, FA	VAN/RIF+CIP	19	No
	2 (139 days)	stage 2	-	-	-	-	-	-	-	-	-	
	3 (13 days)	DAIR, E	Reinfection	6997	<i>S. epidermidis</i>	ST-434	0.59	N	CIP, CLI, OXA, FA, TRI, SUL	VAN/LZD	4	Yes
			Reinfection	6998	<i>S. epidermidis</i>	ST-2	0.215	W	CIP, CLI, OXA, RIF, TRI, SUL	VAN/LZD	4	Yes
K13	1	DAIR, E		7005	<i>S. epidermidis</i>	ST-2	0.318	M	CIP, CLI, OXA, FA, TRI, SUL	VAN/TEI	34	No
	2 (529 days)	stage 1	Relapse	7006	<i>S. epidermidis</i>	ST-2	3.322	S	CIP, CLI, OXA, FA	VAN/LZD	6	Yes, after stage 2
K2	1	stage 1	First-time	6999	<i>S. epidermidis</i>	ST-215	0.073	N	CIP, CLI, OXA, FA, TRI, SUL	VAN/0	13	No
				7000	<i>S. epidermidis</i>	ST-215	0.099	N	CIP, CLI, OXA, FA	VAN/0	13	No
	2 (126 days)	stage 2	-	-	-	-	-	-	-	-	-	
	3 (348 days)	stage 1	Relapse from 6999	7001	<i>S. epidermidis</i>	ST-215	0.055	N	CIP, CLI, OXA, FA, TRI, SUL	VAN/0	8	Yes, after stage 2
K23	1	DAIR, E		7012	<i>S. aureus</i>	ST-630	0.441	M	0	FLX/RIF+CIP	16	No
	2 (157 days)	stage 1	Relapse	7013	<i>S. aureus</i>	ST-630	0.277	M	0	FLX/RIF+CIP	32	No
K29	1	DAIR, E		7018	<i>S. aureus</i>	ST-20	1.140	S	0	VAN/LZD	7	No
				7019	<i>S. epidermidis</i>	ST-215	0.058	N	CIP, CLI, OXA, FA, TRI, SUL	VAN/LZD	7	No
				7020	<i>S. epidermidis</i>	ST.2	0.608	S	CIP, OXA, TRI, SUL	VAN/LZD	7	No
				7021	<i>S. simulans</i>	-	0.275	M	CIP, OXA	VAN/LZD	7	No
	2 (51 days)	DAIR, E	Relapse from 7018	7022	<i>S. aureus</i>	ST-20	0.764	S	0	VAN/RIF+CIP	2	No
	3 (15 days)	stage 1	Relapse from 7020	7023	<i>S. epidermidis</i>	ST-2	0.667	S	CIP, OXA, TRI, SUL	VAN/RIF+CIP	15	Yes, after stage 2
			Relapse from 7020	7024	<i>S. epidermidis</i>	ST-2	0.902	S	CIP, OXA, TRI, SUL	VAN/RIF+CIP	15	Yes, after stage 2
K8	1	DAIR, NE		7002	<i>S. aureus</i>	ST-789	1.821	S	0	VAN/0	0	No
	2 (2 days)	DAIR, E	Reinfection	7003	<i>S. capitis</i>	-	0.411	M	OXA	VAN/RIF+CLI	28	Yes



Supplementary Figure S4. Mean MBEC/MIC-ratios per antimicrobial agent and different degrees of biofilm production. was further divided in subcategories in order to investigate if there were differences between different biofilm degrees. The breakpoints for this new classification were: non-producer ($OD_{570} < 0.120$), weak ($0.120 < OD_{570} < 0.240$), moderate ($0.240 < OD_{570} < 0.480$) and strong ($OD_{570} > 0.480$). *indicates statistical significant difference between the groups with $p < 0.001$.