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Evaluating the potential of novel antimicrobial strategies in a multimodal treatment model for implant-related infections: antibiotics, antimicrobial peptides, bacteriophages, induction heating

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Curriculum Vitae

Ellen Verheul was born on March 4th 1997 in Gouda, the Netherlands. She obtained her VWO diploma at Zuyderzee Lyceum Emmeloord in 2015, and moved to Leiden to start her bachelor's studies Biopharmaceutical Sciences at Leiden University in September 2015. She completed her bachelor research internship at the Department of Biotherapeutics at Leiden Academic Center for Drug Research (LACDR). In addition, Ellen completed the minor in Brain and Cognition at Leiden University and the extracurricular minor Clinical Immunology at the Leiden University Medical Center (LUMC). In February 2019, she enrolled in the research master Biomedical Sciences at Leiden University. Under supervision of dr. Bart van Vlijmen at the Department of Thrombosis and Hemostasis (LUMC), she completed her minor research internship. This research focused on the influence of local tissue factor in directing spontaneous venous thrombosis to a specific area in the mouse head. Further, she completed the master Honours Class "Innovation Health and Well-being through Entrepreneurship." During the master course "Regulation of the Immune Response in Human Disease", she developed her interest in infectious diseases research. Subsequently, Ellen joined the group of dr. Peter Nibbering at the Leiden University Center for Infectious Diseases (LUCID, LUMC) to complete her major research internship. Here, she investigated the effects of two alternative treatment approaches to eradicate bacterial biofilms on metal implants: induction heating and antimicrobial peptides. After obtaining her master's degree in June 2021, she directly started her PhD research in the LUMC at LUCID and the Department of Orthopedics under the supervision of dr. Peter Nibbering (LUCID), dr. Bart Pijls (Department of Orthopedics) and prof. dr. Rob Nelissen (Department of Orthopedics). In her doctoral research, Ellen investigated the effects of bacteriophages, antibiotics, induction heating, antimicrobial peptides, and their combinations on biofilm-associated *Staphylococcus aureus* on metal implant surfaces *in vitro*.

Portfolio

The PhD trajectory - an overview

Ellen's PhD trajectory focused on assessing the effects of alternative strategies to combat difficult-to-treat infections, with a particular emphasis on *Staphylococcus aureus* biofilms associated with metal implant-related infections. During her Master's thesis at the Department of Infectious Diseases, she investigated the effects of non-contact induction heating in combination with the antimicrobial peptide SAAP-148 on persister-enriched *Staphylococcus aureus* biofilms (**Chapter 5**). This work introduced her to biofilm biology, techniques to enrich bacterial biofilms with persisters as well as antimicrobial peptides and induction heating as novel anti-biofilm strategies. This experience shaped her interest in innovative, non-antibiotic treatment approaches to combat metal implant-related infections and provided the foundation for her subsequent PhD research.

Building on this background, Ellen's first PhD project focused on the application of bacteriophages across different stages of the biofilm life cycle (**Chapter 2**). In this context, she utilized various *in vitro* biofilm models to mimic the biofilm life cycle and became familiar with experimental work involving phages. In parallel, she initiated a collaboration with the electron microscopy facility at the LUMC. This collaboration aimed to identify the presence of phages throughout the biofilm by visualizing phage-exposed biofilms cross-sectionally using transmission electron microscopy. This project revealed that phages alone were ineffective at eradicating biofilm-associated bacteria, suggesting the presence of bacterial tolerance mechanisms. She disseminated her findings through a poster presentation at the Gordon Research Conference on Antimicrobial Discovery, as well as through oral presentations at the NCOH Science Café and the PhageNL symposium. These presentations further strengthened her scientific communication skills and visibility within the field. Additionally, Ellen supervised a student for four months and was involved in the laboratory course Infectious Agents and Immunity.

Her next project further expanded on the abovementioned findings by exploring combination therapies involving induction heating, SAAP-148, antibiotics, and phage ISP (**Chapter 6**). In this work, Ellen investigated whether the sequence of these strategies influenced treatment efficacy. She utilized confocal microscopy and microbiological assays to assess the effects of these approaches on biofilms. This project reflected her growing expertise in addressing clinically relevant questions in fundamental research. During this period, she supervised another student for four months and continued her teaching contributions within the Infectious Agents and Immunity laboratory course. She presented her work as a poster at the Thesinge Biofilm Meeting and as an oral presentation at the EORS conference. Notably, she received the best presentation award for the scientific Pecha Kucha presentation for the general public, recognizing her ability to communicate complex topics to a broad audience. In addition, she contributed to the development of the DARTBAC AMR Quartet Game, an educational initiative aimed at raising awareness about antimicrobial resistance among non-specialist audiences. These activities highlight her commitment to science communication and public engagement.

Ellen then continued her research with a translational perspective by investigating whether the effects of phage-antibiotic combinations on biofilms could be predicted using planktonic phase bacteria (**Chapter 4**). This project evaluated the accuracy of a first-line screening tool for selecting appropriate phage-antibiotic combinations to treat biofilm-related infections.

She disseminated the findings of this study through oral presentations at ESCMID Global in 2023 and 2024. Ellen also initiated a study to investigate the effects of phages on immune effector functions of phagocytes. In parallel, she supervised a student for nine months across both projects.

Throughout her PhD, Ellen was actively engaged in team science and collaborative projects within and across institutions. Regarding the latter, Ellen contributed to a review paper on biofilms, small colony variants, and intracellular bacteria in collaboration with the Department of Trauma Surgery in Regensburg. In addition, she contributed to a review on antimicrobial resistance in orthopedics, alongside colleagues from the DARTBAC consortium. Within DARTBAC, she frequently shared data and protocols, thereby contributing to open and transparent research practices. Ellen also took an active role in organizing departmental social events as part of the laboratory's internal activities committee. She co-organized yearly summer schools and consortium meetings for DARTBAC and thereby gained experience in coordinating academic events. As a member of the Young Board of the National Consortium on One Health, she represented early-career researchers and contributed to national-level interdisciplinary events.

In the final phase of her PhD, Ellen initiated a collaborative follow-up project with the M4i institute at Maastricht University to study the mechanisms underlying the earlier-observed tolerance of biofilm-dispersed bacteria to phages (**Chapter 3**). This project integrated proteomic, microbiological, and biochemical analyses to examine this tolerant phenotype at the molecular level. This work marked a shift toward more mechanistic and systems-level investigations and demonstrates Ellen's ability to coordinate interdisciplinary research across institutions. In the final year of her PhD, Ellen also took the initiative to write a research grant proposal.

CRediT statement for the Chapters in this thesis

CRediT table for the thesis of Ellen Verheul																			
Ch.	Type*	Short Title	Conceptualization	Data Curation	Formal Analysis	Funding Acquisition	Investigation	Methodology	Project Administration	Resources	Software	Supervision	Validation	Visualization	Writing – Original Draft	Writing – Review & Editing	Pre-registered	Preprinted	Published with Peer Review
1	Introduction	General introduction																	↘
2	PhD project chapter	Efficacy ISP on the biofilm cycle																	↘
3	PhD project chapter	Phage-tolerance biofilm dispersal																	
4	PhD project chapter	PAC efficacy prediction biofilms																	↘
5	PhD project chapter	NCIH and SAAP-148 on persisters																	↘
6	PhD project chapter	NCIH, antibiotics, SAAP-148 & ISP																	↘
7	Discussion	Summarizing discussion																	

*PhD project chapters are the direct result of the PhD project of the PhD candidate.
Some theses also include Collaboration Chapters, to which the PhD candidate has contributed but fall outside the PhD project.

Dissemination table

1. Verheul M. , Drijfhout J.W., Pijls B.G.,* Nibbering P.H.* (2021) Non-contact induction heating and SAAP-148 eliminate persisters within MRSA biofilms mimicking a metal implant infection. <i>European Cells and Materials</i> . 43: 34-42.	
Chapter in this thesis:	Chapter 5
Publication in peer reviewed journal:	DOI: 10.22203/eCM.v042a03
Conference contributions of the PhD candidate:	Poster presentation Thesinge Biofilm Meeting (2022)
2. van Dun S.C.J., Verheul M. , Pijls B.G., van Prehn J., Scheper H., Galli F., Nibbering P.H., de Boer M.G.J. (2023) Influence of surface characteristics of implant materials on MRSA biofilm formation and effects of antimicrobial treatment. <i>Frontiers in Microbiology</i> . 14: 1145210.	
Publication in peer reviewed journal:	DOI: 10.3389/fmicb.2023.1145210
3. Straub J., Baertl S., Verheul M. , Walter N., Wong R.M.Y., Alt V., Rupp M. (2024) Antimicrobial resistance: Biofilms, small colony variants, and intracellular bacteria. <i>Injury</i> . 55 Suppl 6: 111638.	
Publication in peer reviewed journal:	DOI: 10.1016/j.injury.2024.111638.
4. Verheul M. , Mulder A.A., van Dun S.C.J., Merabishvili M., Nelissen R.G.H.H., de Boer M.G.J., Pijls B.G., Nibbering P.H. (2024) Bacteriophage ISP eliminates <i>Staphylococcus aureus</i> in planktonic phase, but not in the various stages of the biofilm cycle. <i>Scientific Reports</i> . 14(1): 14374.	
Chapter in this thesis:	Chapter 2
Publication in peer reviewed journal:	DOI: 10.1038/s41598-024-65143-9
Conference contributions of the PhD candidate:	- Poster presentation GRC New Antibacterial Discovery and Development (2022) - Oral presentation NCOH Science Café (2024) - Oral presentation mini-symposium PhageNL (2025)
5. van Dun S.C.J., Verheul M. , Pijls B.G., Scheper H., van der Does A.M., Nibbering P.H., de Boer M.G.J. (2025) Effectiveness of clindamycin-based exposure strategies in experimental mature staphylococcal biofilms. <i>Microbiology Spectrum</i> . 13(3): e0194724.	
Publication in peer reviewed journal:	DOI: 10.1128/spectrum.01947-24

6. Verheul M., Wagenmakers A.A., Nelissen R.G.H.H., Nibbering P.H., Pijls B.G. (2025) Induction heating combined with antibiotics or SAAP-148 effectively reduces biofilm-embedded <i>Staphylococcus aureus</i> on a fracture-related implant mimic. <i>Bone and Joint Research</i>. 14(5): 485-494	
Chapter in this thesis:	Chapter 6
Publication in peer reviewed journal:	DOI: 10.1302/2046-3758.145.BJR-2024-0341.R1
Conference contributions of the PhD candidate:	Oral presentation EORS (2025)
7. van Agtmaal J.L., Verheul M., Vonken L., Helsen K, Vargas Guerrero M.G., van Hoogstraten S.W.G., Hurck B.J., Pilla G., Trinh I., de Bruijn G.J., Calum H.P., de Boer M.G.J., Pijls B.G., Arts J.J.C. (2025) Antimicrobial resistance in orthopedics: microbial insights, clinical impact, and the necessity of a multidisciplinary approach - a review. <i>Acta Orthopaedica</i>. 96: 555-568	
Chapter in this thesis:	Chapter 1 (partly)
Publication in peer reviewed journal:	DOI: 10.2340/17453674.2025.43477
8. Verheul M., Schonkeren-Ravensbergen E., Bus E.M., de Boer M.G.J., Nelissen R.G.H.H., Pijls B.G.,* Nibbering P.H.* (2026) Efficacy prediction of bacteriophage-antibiotic combinations against <i>Staphylococcus aureus</i> biofilms using planktonic bacteria. <i>Frontiers in Microbiology</i>. 16: 1706083	
Chapter in this thesis:	Chapter 4
Publication in peer reviewed journal:	DOI: 10.3389/fmicb.2025.1706083
Conference contributions of the PhD candidate:	- Oral presentation ESCMID Global (2023) - Oral presentation ESCMID Global (2024)
9. Kamphof, R., Verheul M., Nelissen R.G.H.H., de Boer M.G.J., Pijls B.G. (2026) The effect of elevated physiological temperatures on bacterial survival and antibiotic susceptibility in an <i>in vitro</i> infection prevention model. <i>Frontiers in Microbiology</i>. 17: 1830056	
Publication in peer reviewed journal:	DOI: 10.3389/fmicb.2026.1830056

* These authors contributed equally to this research

Overview of completed courses and other training

Mandatory activities			
<i>Year, month</i>	<i>Description</i>	<i>Title</i>	<i>Hours</i>
2021, Nov	Course	Leiden University Onboarding Programme Inform & Connect	5
2022, Jan - Feb	Course	Basic Methods and Reasoning in Biostatistics	42
2023, Nov - Dec	Course	eBROK (Basic Course Rules and Organisation for Clinical Researchers)	42
2025, Jan	Workshop	Workshop Scientific Conduct for PhDs	5

Scientific courses, workshops, and other training activities			
<i>Year, month</i>	<i>Description</i>	<i>Title</i>	<i>Hours</i>
2021, Nov	Meeting	NWA-DARTBAC Consortium Meeting	16
2022, Jan	Course	LIFI PhD Online Course Infection and Immunity	56
2022, Apr	Meeting	NWA-DARTBAC Consortium Meeting	16
2022, May	Conference	Thesinge Biofilm Meeting	16
2022, May	Meeting	NWA-DARTBAC Young Scientist Meeting	18
2022, Jul	Conference	Phage Futures Europe	14
2022, Jul	Conference	GRC New Antibacterial Discovery and Development	40
2022, Nov	Meeting	NWA-DARTBAC Consortium Meeting	16
2023, Apr	Conference	ESCMID Global	36
2023, May	Meeting	NWA-DARTBAC Young Scientist Meeting	24
2023, Nov	Meeting	NWA-DARTBAC Consortium Meeting	16
2024, Apr	Meeting	NWA-DARTBAC Consortium Meeting	16
2024, Apr	Conference	ESCMID Global	32
2024, May	Meeting	NCOH Annual Scientific Meeting	8
2024, Jun	Meeting	NWA-DARTBAC Young Scientist Meeting	16
2024, Sep	Conference	EORS	24
2024, Nov	Meeting	NWA-DARTBAC Consortium Meeting	14
2024, Dec	Symposium	Minisymposium PhageNL	4
2025, Apr	Meeting	NWA-DARTBAC Consortium meeting 2025	12

Transferable skills courses, workshops, and other training activities			
<i>Year, month</i>	<i>Description</i>	<i>Title</i>	<i>Hours</i>
2021 - 2022	Teaching activity	BW2 Infectious Agents and Immunity	25
2021, Nov - Dec	Course	Communication in Science for PhDs	35
2021, Dec - 2024, Sep	Other	Member of NWA-DARTBAC Young Scientist Committee	60
2022, Apr	Course	Mindfulness	8
2022, Mar - Jul	Teaching activity	Supervision of student	28
2023, Mar - Jul	Teaching activity	Supervision of student	28
2023, Sep - 2024, Jun	Teaching activity	Supervision of student	72
2024, Oct	Workshop	Workshop Theatrical Presenting	3
2024, Apr - 2026, Jul	Other	Board member of Young NCOH	56

Dissemination, acknowledgement, esteem, and other relevant scientific activities

Other scientific activities related to this thesis			
<i>Year, month</i>	<i>Description</i>	<i>Title</i>	<i>Linked to chapter(s)</i>
2022, May	Presentation	Poster presentation Thesinger Biofilm Meeting	5
2023, Jul	Presentation	Poster presentation GRC New Antibacterial Discovery and Development	2, 4
2024, Apr	Presentation	Oral presentation ESCMID Global	4
2023, Nov	Presentation	Presentation NCOH Science Café	2
2023, Nov	Awards and Prizes	Pecha Kucha contest: best scientific presentation for the general public	5, 6
2024, Apr	Presentation	Oral presentation ESCMID Global	4
2024, Sep	Presentation at LUMC	Oral presentation LUCID meeting	2, 3
2024, Dec	Presentation	Oral presentation minisymposium PhageNL	2
2025, Feb	Presentation	Invited speaker at EURESTOP/STIMULUS Young Researchers & Innovators Forum	5, 6
2025, Jun	Grant application		

Dankwoord

“Ik ga nooit een PhD doen” - Ellen Verheul (2018)

Diezelfde ik kan het haast niet geloven dat ik bij het schrijven van mijn dankwoord ben beland. Met veel trots en plezier kijk ik terug op een waardevolle en intensieve periode waarin ik mijn promotieonderzoek heb voltooid. Dit had ik natuurlijk nooit alleen kunnen doen: hoewel ik hieronder mensen in het bijzonder noem, zou ik graag iedereen willen bedanken die aan deze reis heeft bijgedragen.

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