M U N I

COMMENTARY TO HABILITATION THESIS[1]

Mobile Genetic Elements: Friends or Foes of Staphylococci?

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This habilitation thesis addresses the dual role of mobile genetic elements (MGEs) in staphylococci: their contribution to pathogenicity and their application in the treatment of difficult-to-treat infections. The first part deals with the role of MGEs as sources of factors that determine virulence and antimicrobial resistance, which bacteria exploit to enhance their fitness. On the other hand, the second part presents how to utilize bacterial viruses (phages) to combat pathogenic bacterial strains, thereby improving or supplementing traditional therapeutic approaches.

The thesis is composed of 17 selected peer-reviewed original research articles and 1 review that highlight my contributions to the field, including research on the characterization of antimicrobial resistance- and virulence-associated plasmids, the role of prophages in bacterial virulence and anti-phage defense, and the investigation of horizontal gene transfer among staphylococcal species. Our findings revealed a new, distinct lineage of large exfoliative toxin B-encoding plasmids, a previously unknown abortive system of prophage origin, and provided new insights into the variability of temperate phages and plasmids of *Staphylococcus aureus*. With my colleagues, including students I supervised, we investigated the mobilization of plasmids and chromosomal islands of *Staphylococcus epidermidis* by temperate bacteriophages. We were the first to compare their genomics and provide evidence of a phage-inducible chromosomal island transfer in this species. Thus, we have substantially contributed to knowledge of interactions between phages and phage-inducible chromosomal islands that shape the dynamics of the staphylococcal mobilome. One of my major contributions is the innovative application of long-read sequencing to characterize complete encapsidated genophores, providing new insight into generalized and lateral transduction and the effects of environmental stress on mobilome transmission.

Furthermore, I focused on the phenotypic and genomic characterization of lytic phages, including their therapeutic potential and safety. We demonstrated that phages of the genus *Kayvirus* are highly suitable for therapeutic use due to their broad host range, safety,

[1] The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant's contribution in terms of both quality and content.

and ability to suppress the spread of bacterial MGEs. An important part of my research in the field of phage therapy is the exploitation of synergistic effects when combining phages and antibiotics, as well as other translational approaches to improve strategies against difficult-to-treat staphylococcal infections. In addition to the enhanced lytic efficacy of phage-antibiotic combinations, we have also described their effects on changes in the susceptibility of staphylococcal strains, the suppression of biofilm growth, and the inhibition of antimicrobial resistance emergence. Through our collaboration with structural virologists, we have uncovered the relationships between genomics and the mechanisms of phage infection. Together, these studies connect fundamental research of bacterial evolution with the development of novel antimicrobial strategies and the assessment of their safety.

I am the first, co-first, or corresponding author of six of the presented articles, making the core of this thesis. My contribution to each work is detailed in the tables below. The manuscripts are arranged chronologically from the most recent, and the Journal Impact Factor (JIF) and JIF quartile or decile ranking are provided based on data from Web of Science (Journal Citation Reports, Clarivate).

[1][2] Pantůček R, Doškař J, Boštík J, Kocourková D, Petrzik K, Koptíková J, Benešík M, Plevka P, Füzik T, Mašlaňová I, Botka T, Nepeřený J, Zeller D, Kuntová L, Snopková K, Hrala M, Chumchalová J, Holý D, Moša M (2026) Past and present phage therapy research and practice in the Czech Republic – contribution to phage therapy in Europe. *Virology Journal*. https://doi.org/10.1186/s12985-026-03296-x (JCR 2025: IF = 4.0; Q1 – Virology)
Contribution: Review design (the topics of phage-antibiotic synergy, long-read sequencing, and partially therapeutic kayviruses), figure design, data analysis, participation in manuscript draft and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
N/A	10	10	10

[2] Bíňovský J, Šiborová M, Zlatohurska M, Nováček J, Bárdy P, Baška R, Škubník K, Botka T, Benešík M, Pantůček R, Tripsianes K, Plevka P (2026) Conformational changes of the baseplate regulating tail contraction of *Staphylococcus* phage 812. *EMBO Journal* 45:5514–5536. https://doi.org/10.1038/s44318-026-00834-9 (JCR 2025: IF = 8.4; Q1 – Biochemistry & Molecular Biology)
Contribution: Research design (correlation of sequencing and structural data), investigation (genomic analysis), participation in manuscript draft and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
5	-	10	10

[2] Bibliographic record of a published scientific result, which is part of the habilitation thesis.

[3] Siváková A, Figallová E, Botka T (corresp. author), Vacek L, Tkadlec J, Vrbský J, Osowski M, Petráš P, Polaštík Kleknerová D, Dvořáčková M, Urbanová P, Pantůček R, Růžička F (2026) The combination of mupirocin and *Kayvirus* broadens the decolonization effect against *Staphylococcus aureus*. *Applied Microbiology and Biotechnology* 110:157. https://doi.org/10.1007/s00253-026-13814-0 (JCR 2025: IF = 4.7; Q2 – Biotechnology & Applied Microbiology)

Contribution: Research design, investigation (genome sequencing, assembly, and annotation, genomic and proteomic analysis), data analysis, figure design, manuscript draft, writing, and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	80	60	70

[4] Cieniková Z, Nováček J, Šiborová M, Popelářová B, Füzik T, Botka T, Benešík M, Bárdy P, Pantůček R, Plevka P (2026) Genome anchoring, retention, and release by neck proteins of *Staphylococcus* phage 812. *Communications Biology* 9:199. https://doi.org/10.1038/s42003-025-09477-8 (JCR 2025: IF = 5.8; D1 – Biology)

Contribution: Research design (residual capsid DNA detection), investigation (DNA sequencing, assembly and annotation, genomic analysis), figure design, manuscript draft, writing, and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	-	10	15

[5] Botka T, Smetanová S, Vinco A, Kučerová E, Rovňáková K, Siváková A, Mašlaňová I, Pantůček R (2025) Virion content unpacked by long-read sequencing: stress-induced changes in transmitted staphylococcal mobilome due to phage-satellite interactions. *Nucleic Acids Research* 53:gkaf1165. https://doi.org/10.1093/nar/gkaf1165 (JCR 2025: IF = 15.0; D1 – Biochemistry & Molecular Biology)

Contribution: Research design, investigation (virion preparation, virion and bacterial DNA isolation, sequencing, assembly, and annotation, genomic analysis, participation in phage-host system preparation), data analysis, figure design, manuscript draft, writing, and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
60	90	80	90

[6] Komárková M, Benešík M, Procházková T, Vinco A, Laichmanová M, Smetanová S, Jelínek P, Moša M, Botka T, Šoóš M, Pantůček R (2025) Compatibility of antimicrobial preservatives with therapeutic bacteriophages of the genera *Pbunavirus* and *Kayvirus*. *European Journal of Pharmaceutics and Biopharmaceutics* 214:114781. https://doi.org/10.1016/j.ejpb.2025.114781 (JCR 2025: IF = 5.0; Q1 – Pharmacology & Pharmacy)

Contribution: Research design (Kayvirus stability), data analysis, and manuscript revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
-	20	10	15

[7] Šimečková H, Bárdy P, Kuntová L, Macháčová E, Botka T, Bíňovský J, Houser J, Farka Z, Plevka P, Pantůček R, Mašlaňová I (2025) CRISPR-Cas10-assisted structural modification of staphylococcal *Kayvirus* for imaging and biosensing applications. *ACS Synthetic Biology* 14:2979–2986. https://doi.org/10.1021/acssynbio.5c00387 (JCR 2025: IF = 4.5; Q1 – Biochemical research methods)

Contribution: Research design (sequencing approach for tagged phage DNA analysis), investigation (genome sequencing, assembly, annotation, and analysis), data interpretation, figure design, participation in manuscript writing and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
15	-	10	10

[8] Mašlaňová I, Kovařovic V, Botka T, Švec P, Sedláček I, Šedo O, Finstrlová A, Neumann-Schaal M, Kirstein S, Schwendener S, Staňková E, Rovňáková K, Petráš P, Doškař J, Perreten V, Pantůček R (2025) Evidence of *in vitro mecB*-mediated β-lactam antibiotic resistance transfer to *Staphylococcus aureus* from *Macrococcus psychrotolerans* sp. nov., a psychrophilic bacterium from food-producing animals and human clinical specimens. *Applied and Environmental Microbiology* 91:e0165224. https://doi.org/10.1128/aem.01652-24 (JCR 2025: IF = 4.2; Q2 – Biotechnology & Applied Microbiology)

Contribution: Research design (linear plasmid identification approach), investigation (DNA isolation, sequencing, assembly, and annotation, genomic analysis), data analysis, figure design, participation in manuscript writing and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	-	15	20

[9] Obořilová R, Kučerová E, Botka T, Vaisocherová-Lísalová H, Skládal P, Farka Z (2025) Piezoelectric biosensor with dissipation monitoring enables the analysis of bacterial lytic agent activity. *Scientific Reports* 15:3419. https://doi.org/10.1038/s41598-024-85064-x (JCR 2025: IF = 4.9; Q1 – Multidisciplinary Sciences)

Contribution: Research design (methodology, phage-antibiotic synergy), participation in phage preparation, data analysis, figure design, participation in manuscript writing and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
5	50	20	50

[10] Vacek L, Polaštík Kleknerová D, Lipový B, Holoubek J, Matysková D, Černá E, Brtníková J, Jeklová E, Kobzová Š, Janda L, Lišková L, Diabelko D, Botka T, Pantůček R, Růžička F, Vojtová L (2024) Phage therapy combined with Gum Karaya injectable hydrogels for treatment of methicillin-resistant *Staphylococcus aureus* deep wound infection in a porcine model. *International Journal of Pharmaceutics* 660:124348. https://doi.org/10.1016/j.ijpharm.2024.124348 (JCR 2024: IF = 5.2; Q1 – Pharmacology & Pharmacy)

Contribution: Participation in research design (methodology, conceptualization), phage preparation, data analysis, and manuscript revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
5	10	10	15

[11] Kuntová L, Mašlaňová I, Obořilová R, Šimečková H, Finstrlová A, Bárdy P, Šiborová M, Troianovska L, Botka T, Gintar P, Šedo O, Farka Z, Doškař J, Pantůček R (2023) *Staphylococcus aureus* prophage-encoded protein causes abortive infection and provides population immunity against kayviruses. *mBio* 14:e0249022. https://doi.org/10.1128/mbio.02490-22 (JCR 2023: IF = 5.1; Q1 – Microbiology)

Contribution: Research design (participation in methodology, fused gene product function), investigation (DNA sequencing, assembly, and annotation, genomic analyses), figure design, and manuscript revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	20	10	15

[12] Kovařovic V, Finstrlová A, Sedláček I, Petráš P, Švec P, Mašlaňová I, Neumann-Schaal M, Šedo O, Botka T, Staňková E, Doškař J, Pantůček R (2023) *Staphylococcus brunensis* sp. nov. isolated from human clinical specimens with a staphylococcal cassette chromosome-related genomic island outside of the *rlmH* gene bearing the *ccrDE* recombinase gene complex. *Microbiology Spectrum* 11:e0134223. https://doi.org/10.1128/spectrum.01342-23 (JCR 2023: IF = 3.7; Q2 – Microbiology)

Contribution: Research design (sequencing and analysis methodology), investigation (DNA sequencing, assembly and annotation, genomic analyses), and manuscript revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
15	15	10	15

[13] Fišarová L*, Botka T*, Du X*, Mašlaňová I, Bárdy P, Pantůček R, Benešík M, Roudnický P, Winstel V, Larsen J, Rosenstein R, Peschel A, Doškař J (2021) *Staphylococcus epidermidis* phages transduce antimicrobial resistance plasmids and mobilize chromosomal islands. *mSphere* 6:e00223-21. https://doi.org/10.1128/mSphere.00223-21 *co-first authors (JCR 2021: IF = 5.029; Q2 – Microbiology)

Contribution: Research design (sequencing, analysis, and transduction methodology), investigation (phage and bacterial DNA isolation, plasmid isolation, sequencing, assembly, and annotation, genomic and proteomic data analysis, lysogenization, participation in transduction experiments, and phage growth characteristics), data analysis, figure design, manuscript draft, writing, and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	30	50	50

[14] Botka T, Pantůček R, Mašlaňová I, Benešík M, Petráš P, Růžičková V, Havlíčková P, Varga M, Žemličková H, Koláčková I, Florianová M, Jakubů V, Karpíšková R, Doškař J (2019) Lytic and genomic properties of spontaneous host-range *Kayvirus* mutants prove their suitability for upgrading phage therapeutics against staphylococci. *Scientific Reports* 9:5475. https://doi.org/10.1038/s41598-019-41868-w (JCR 2019: IF = 3.998; Q1 – Multidisciplinary Sciences)

Contribution: Research design, investigation (phage lysate preparation, phage DNA isolation, PCR design and conduct, participation in strain genotyping, phage susceptibility testing, and sequencing, genome assembly and annotation, variant calling, genome physical end determination, promoter prediction, genomic and proteomic comparative analysis), figure design, manuscript draft, writing, and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	40	80	70

[15] Fišarová L, Pantůček R, Botka T, Doškař J (2019) Variability of resistance plasmids in coagulase-negative staphylococci and their importance as a reservoir of antimicrobial resistance. *Research in Microbiology* 170:105–111. https://doi.org/10.1016/j.resmic.2018.11.004 (JCR 2019: IF = 3.217; Q2 – Microbiology)

Contribution: Research design (plasmid sequence analysis), investigation (plasmid sequence assembly, annotation, and comparative analysis, participation in plasmid content determination), figure design, manuscript draft, writing, and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	20	40	20

[16] Botka T, Růžičková V, Svobodová K, Pantůček R, Petráš P, Čejková D, Doškař J (2017) Two highly divergent lineages of exfoliative toxin B-encoding plasmids revealed in impetigo strains of *Staphylococcus aureus*. *International Journal of Medical Microbiology* 307:291–296. https://doi.org/10.1016/j.ijmm.2017.05.005 (JCR 2017: IF = 3.298; Q2 – Microbiology)

Contribution: Research design, investigation (bacterial strain and plasmid PCR typing, isolation and restriction profiling of plasmids, PCR design, plasmid sequence assembly and annotation, comparative genomic and phylogenetic analysis), data analysis, figure design, manuscript draft, writing, and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
90	50	90	80

[17] Botka T, Růžičková V, Konečná H, Pantůček R, Rychlík I, Zdráhal Z, Petráš P, Doškař J (2015) Complete genome analysis of two new bacteriophages isolated from impetigo strains of *Staphylococcus aureus*. *Virus Genes* 51:122–131. https://doi.org/10.1007/s11262-015-1223-8 (JCR 2015: IF = 1.285; Q4 – Genetics & Heredity)

Contribution: Research design, investigation (phage isolation, phage DNA isolation, PCR typing, restriction analysis, DNA sequencing, assembly, annotation, comparative genomic

analysis, lysogenization, PFGE analysis, phage virion purification), data analysis, figure design, manuscript draft, writing, and revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
90	20	90	60

[18] Tkadlec J, Vařeková E, Pantůček R, Doškař J, Růžičková V, Botka T, Fila L, Melter O (2015) Characterization of *Staphylococcus aureus* strains isolated from Czech cystic fibrosis patients: High rate of ribosomal mutation conferring resistance to MLS(B) antibiotics as a result of long-term and low-dose azithromycin treatment. *Microbial drug resistance* 21:416–423. https://doi.org/10.1089/mdr.2014.0276 (JCR 2015: IF = 2.529; Q2 – Infectious Diseases)
Contribution: Investigation (bacterial DNA isolation, PCR typing of virulence and antimicrobial resistance determinants, participation in MLST and spa typing), manuscript revision.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	-	5	-