







PHAGE THERAPY OF CRITICAL BACTERIAL INFECTIONS: SINGLE-CENTER EXPERIENCE

Evgenii Rubalskii^{1,2,3}, Stefan Ruemke^{1,2,3}, Christina Salmoukas^{1,2,3}, Karin Burgwitz^{1,2,3}, Axel Haverich^{1,2,3}, Christian Kuehn^{1,2,3}

National Center for Phage Therapy, Hannover Medical School, Hannover, Germany; Department of Cardiac, Thoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany; Slower Saxony Centre for Biomedical Engineering, Implant Research and Development, Hannover, Germany

Introduction

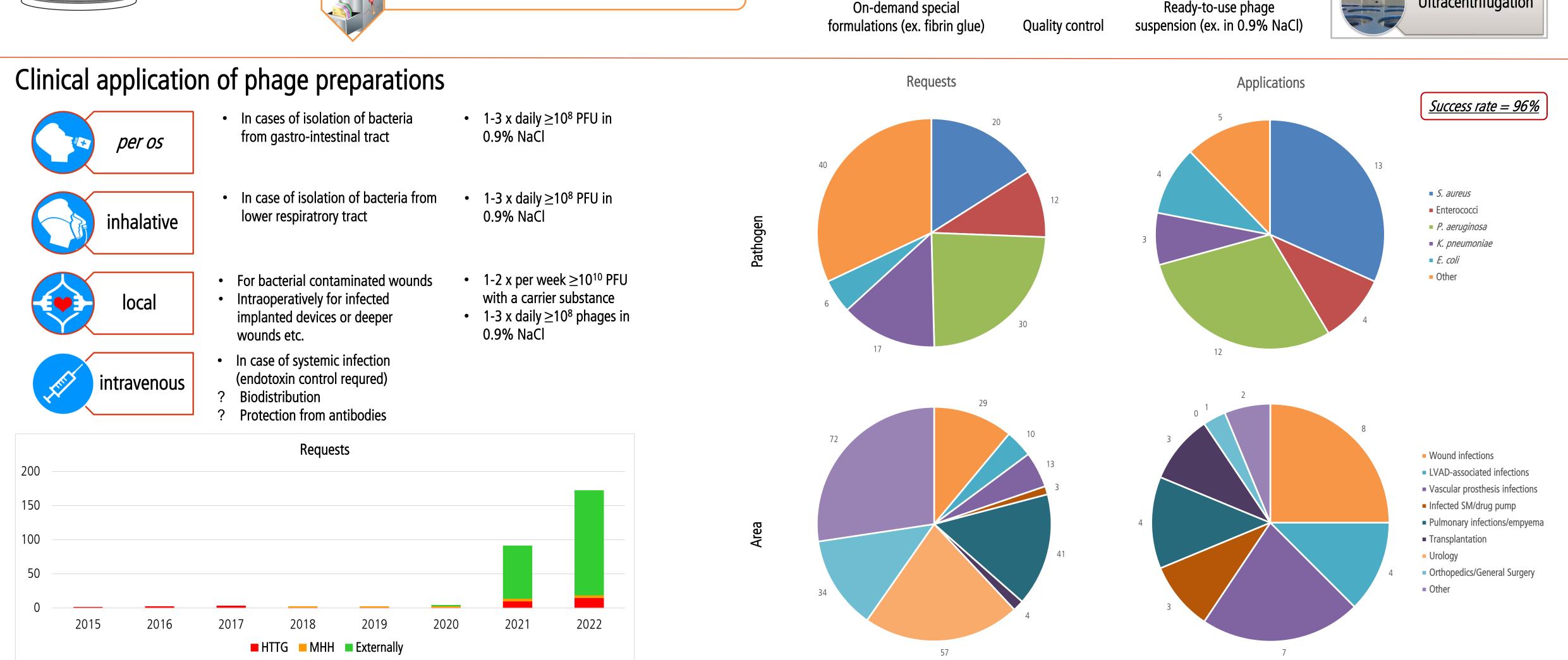
The growing need for the treatment of infections caused by multidrug-resistant pathogens is forcing both clinicians and potential patients to look for alternative therapeutic agents. Currently, phage therapy is considered as an effective alternative or addition to standard antibiotic therapy in cases of critical infections for patients.

However, given the growing popularity of this approach and only emerging international legislation, timely and adequate recommendations for the rational use of bacteriophages for therapeutic purposes are required. On the basis of the Hannover Medical School, we organized the National Center for Phage Therapy.

Patient with an infection caused by multi- or panresistant bacteria Microbiological isolation and identification of the pathogen Selection and preparation of the suitable bacteriophages Clinical application of the phages

Phagogram Soft agar overlay ++ +++ with bacteria and phage Agar plate Negative (no plaques) sample (10× dilutions) Positive (plaques ++++ countable) Plaque assays Liquid media assays Spot-test on bacterial lawn at MOI < 1 Time: 8-18 hours Time: 4-48 hours Time: 4-18 hours

Phage isolation and characterization Preparation of bacteriophages NGS De-novo phage genome assembly with high accuracy **PEG** precipitation Master Phage Genome annotation Microfiltration Affinity columns Screening of Separate amplification of environmental samples bacteriophage strains on solid media Typical titer ≥10¹¹ PFU/mL Assumption of a phage life cycle **PHACTS** Ultrafiltration \geq 3× Selection of single ✓ Phage titer plaques ✓ Sterility • Confirmation of absence of known of homologues ✓ Endotoxin level **Amplification** integrases, antibiotic resistance and virulence factors ✓ Phage identity Ultracentrifugation On-demand special Ready-to-use phage formulations (ex. fibrin glue) suspension (ex. in 0.9% NaCl) **Quality control**



Conclusions

- 1. Well-known phagogram approaches are robust and easy to implement. However new methods with better scalability and timing are needed;
- 2. Whole process from phage isolation to clinical application is feasible for a singe clinical center including modern phage preparation standards;
- 3. Individualized phage therapy is an emerging antibacterial solution with high success rate if appropriate indications and dosage are selected.

MHH
HTTG OE 6210
Carl-Neuberg-Straße 1, 30625 Hannover
Rubalskii.Evgenii@mh-hannover.de
www.mhh.de/nzpt/