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Clinical Phage Microbiology: A Suggestion Framework and Recommendations for Phage Therapy *In vitro* Matching Steps

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Introduction

Phage therapy is a promising treatment for bacterial infections that are resistant to conventional antibiotics. A critical component of this approach is the appropriate matching of bacteriophages and antibiotics to the bacterial target based on the clinical setting. However, there is currently little consistency in the protocols used for laboratory evaluation of bacteriophages intended for antibacterial treatment. In this Personal View, we propose a framework for matching appropriate bacteriophage-based treatments in clinical microbiology laboratories. This framework, dubbed Clinical Phage Microbiology, is based on current phage treatment research. Furthermore, we discuss special cases that may necessitate additional relevant evaluation, such as bacteriophage interactions with the host immune response, biofilm-associated infections, and polymicrobial infections [1].

Description

The susceptibility profile of isolated pathogens, as determined by manual or automated methods, can be used to guide antimicrobial selection for bacterial infectious diseases. The European Committee on Antimicrobial Susceptibility Testing and the US Clinical and Laboratory Standards Institute are currently in charge of antimicrobial susceptibility testing, which has evolved significantly over the last several decades. Methods for each antimicrobial agent are defined to ensure reliability and reproducibility in clinical practise [2].

Phage therapy (the use of bacteriophages as antimicrobial agents) has emerged as a promising treatment option for chronic and resistant bacterial infections. A growing number of reports have described bacteriophage clinical use, which is typically permitted by regulators on compassionate grounds. However, various phage matching methods have been used for this clinical use, typically testing only a few aspects of the phage treatment's suitability. Several papers have described important phage characteristics to consider during treatment design and administration, such as plaque morphology and lysis curve shape. Despite is a standardised protocol for clinical laboratory evaluation of bacteriophages is required due to variations in current clinical practise.

The bacteriophages in the banks should be thoroughly studied. To begin, full genome sequencing is required to ensure the absence of potentially harmful genes such as virulence factors, toxin-producing genes, and antimicrobial resistance genes, as well as to determine phage phylogeny. Furthermore, whether a bacteriophage has lysogenic or only lytic properties can be determined bioinformatically. (eg, by evaluation of their homology to prophages in bacterial genomes).The phage formulations available for clinical use in banks should be produced in accordance with Good Manufacturing Practices. Several publications have outlined the key aspects of GMP for phage production18 and quality control, as well as phage purification protocols. These purification protocols include testing the final preparations for bacteriophage identity, viability, potency, and purity.

The plaque assay is one of the most well-established methods for determining phage efficacy,28 and it has been used in several clinical reports. Using the double-layer agar method, phage suspensions are spotted onto bacterial lawns and the growth inhibition areas, or plaques, are evaluated. Because it only requires a simple phage spotting procedure, this method may be well suited for routine and simultaneous testing of multiple candidate bacteriophages and bacterial isolates. Furthermore, phage titres can be determined by spotting serial dilutions of a bacteriophage suspension and counting plaqueforming units [3].

When selecting bacteriophages for treatment with the plaque assay, several parameters should be considered. Bacteriophages with the clearest and largest areas should be prioritised. Furthermore, expanding plaques may indicate the bacteriophage's ability to lyse non-dividing cells. Bacterial colonies within plaques, on the other hand, can indicate pre-existing resistance, and turbid plaques can indicate lysogenicity29 or efficient defence mechanisms of the target bacteria. When tested on different bacterial isolates, phage morphology and titre can differ. As a result, we believe that phage titre reports should always include the tested bacterial target [4,5].

Conclusion

In this Personal View, we describe a new pipeline called Clinical Phage Microbiology, which aims to develop laboratory guidelines to support clinical phage therapy. This concept considers bacteriophage variability, emphasising the importance of evaluating them in vitro using a combination of several recognised techniques rather than theoretical compatibility. We concentrated on bacteriophages and avoided discussing several related topics, such as the use and application of phagederived proteins, which can be considered conventional antibiotics in many ways. In contrast to the commonly reported approach, we believe that bacteriophages containing genes associated with lysogenicity should be evaluated for clinical use.

Acknowledgement

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Conflict of Interest

None.

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