# Bacteriophages for targeted therapy of multidrug infections

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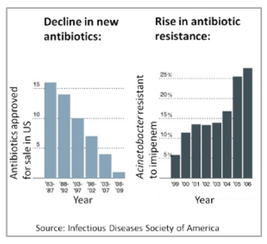
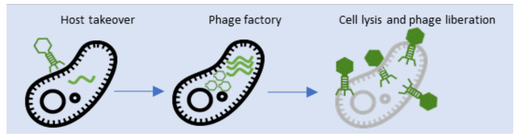
The rise in **multiple antibiotic resistance** (MDR) and consequent failure of traditional antibiotic therapy against main bacterial pathogens call for the implementation of alternative approaches not solely based on antibiotics to fight bacterial infections.




**Bacteriophages** (phages) are viral predators of bacteria. Phages are able to kill bacteria by entering the cell and hijacking bacterial metabolism to reproduce themselves. Newly formed viral particles leave the cell by bursting it, instantly killing the bacterium (lytic cycle).

**LYTIC CYCLE**




**ANTIBIOTICS**

- broad spectrum; easy to use (defined pharmacokinetics); regulated
- resistance 'easy to acquire, hard to lose'; toxic; collateral damage

**BACTERIOPHAGES**

- specialised; abundant; self amplifying; no collateral damage; safe
- unregulated; difficult kinetics/dynamics; unspecified immune response

**ANTIBIOTICS + PHAGES together to target AMR pathogens**

- Severe multidrug resistant bacterial infections and disease outbreaks are often caused by specific pathogenic clones with global spread.

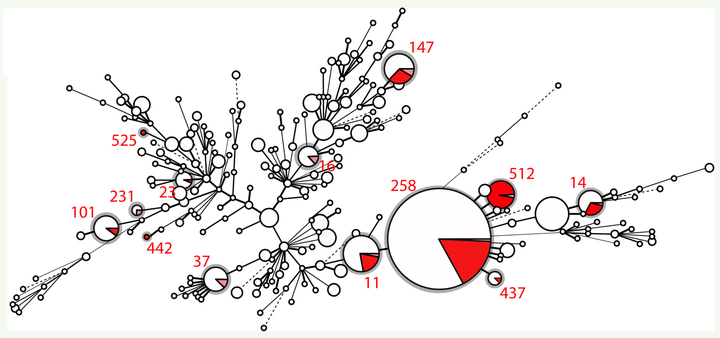
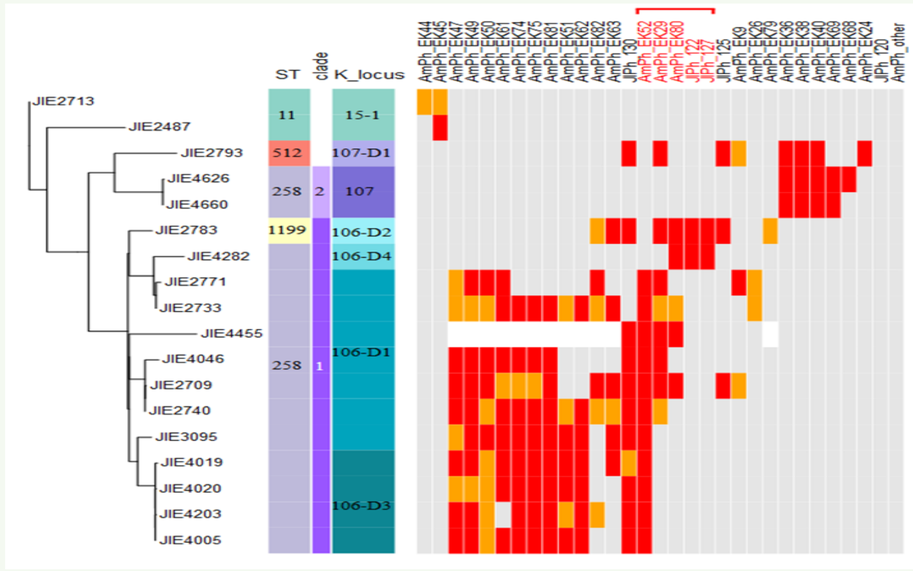



**Figure 1. GLOBAL PREVALENCE of *Klebsiella pneumoniae* CLONAL GROUPS [1].** Sequence type (ST) 258, 11 and 512 are closely relate and belong to clonal group (CC) 258.

- Local epidemiology however can be defined by predominance of specific subtypes within these clonal populations.
- Most bacteriophages are extremely targeted, often attacking only specific strain subtypes.

**Figure 2. *Klebsiella pneumoniae* CC258 in Australia and differential bacteriophage susceptibility of different subtypes [2].**




- For effective therapy, mixes (cocktails) of phages with varying specificities but synergistic activity can be prepared to eliminate specific infections.


**Figure 3. ENHANCED CLEARANCE using PHAGE COMBINATIONS.** We tested the ability of four bacteriophages individually and in combination (cocktail) to supress bacterial growth in liquid media over 18 h. The cocktail outperformed each individual phage at all doses (MOI=multiplicity of infection i.e. phage/bacteria ratio).

- Bacterial response to phage attack leads to mutations that re-sensitize the bacteria to antibiotics. Selected bacteriophage+antibiotic combinations provide clearance of antibiotic resistant bacterial populations.

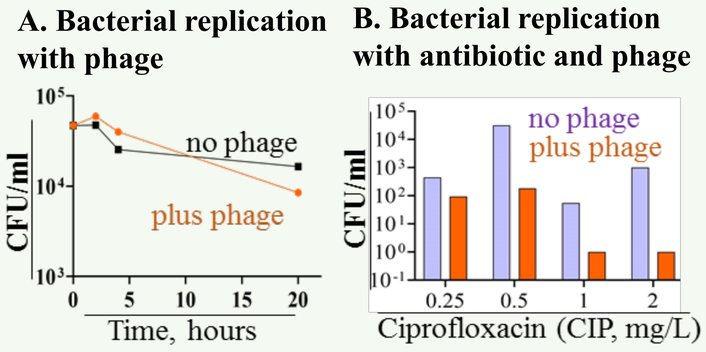



**Figure 4. BACTERIAL REPLICATION in the PRESENCE of ANTIBIOTIC and PHAGE. A.** Presence of phage decreases bacterial load (CFU/mL). **B.** Bacteria resistant to ciprofloxacin are susceptible to combinations of antibiotic and phage.

- We have tested bacteriophage therapy protocols at Westmead Hospital in patients with severe disease unresponsive to antibiotic treatment alone [3,4].

**Figure 5. MULTI-MODAL THERAPEUTIC PHAGE MONITORING** is required to understand phage clearance by immune system.

**Figure 6. COMBINED PHAGE-ANTIBIOTIC THERAPY** is linked to successful clinical outcomes.

**Figure 7. REGULATORY FRAMEWORKS.** Compassionate use only for phages is at present accepted by the regulators. Randomised clinical trials are needed to determine *in vivo* efficacy.

**Fig. 5.**

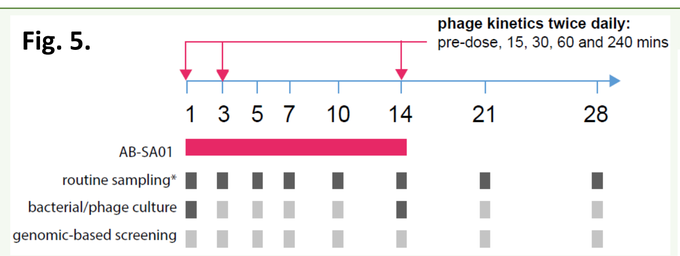



**Fig. 6.**

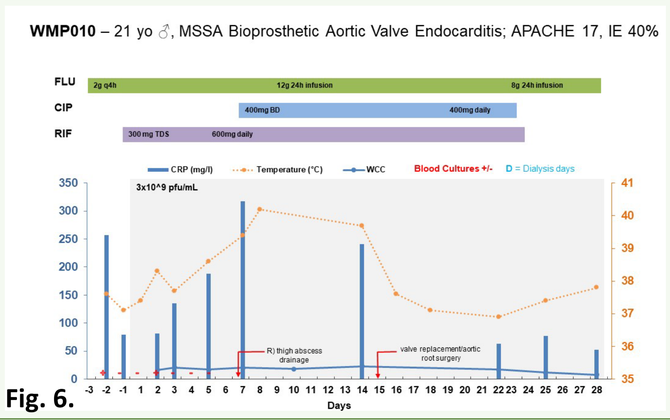
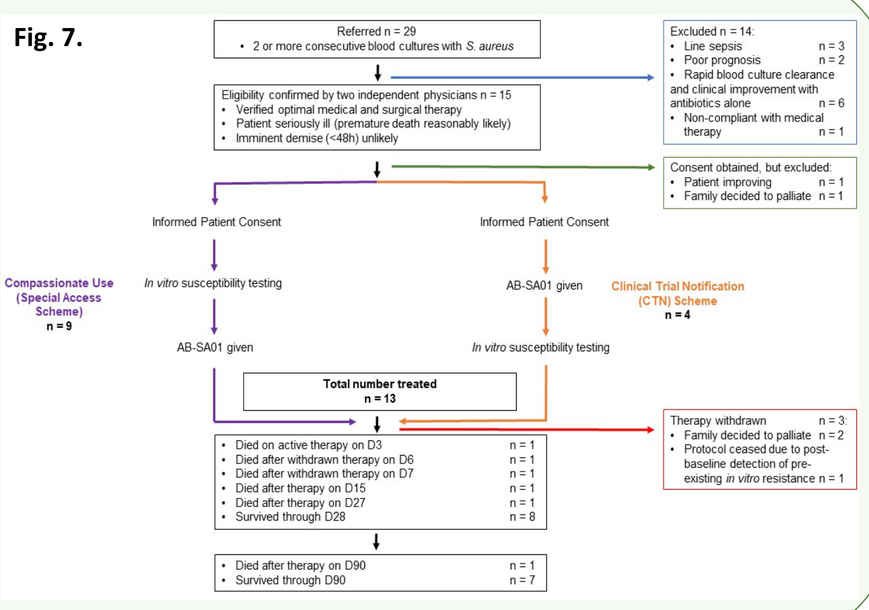



**Fig. 7.**




Bacteriophage therapy is a promising adjunct to standard antibiotic treatment, particularly against multidrug resistant infections. Our understanding of bacteriophage epidemiology and biology is improving, but routine implementation of phage therapy in the clinic requires further research into underlying mechanisms of phage-bacteria interactions and commitment to randomized clinical trials in order to deliver robust and reliable therapeutic outcomes.

**References.** **1.** Fajardo-Lubián A et al., 2019 PLoS Pathogens 15:e1007218; **2.** Venturini et al., 2020 FASEB J 34:10801-17; **3.** Khawaldeh A et al., 2011 JMM 60:1697-700.; **4.** Petrovic Fabijan A et al., 2019 Nat Microbiol 5:465-72.