Bacteriophages and endolysins to tackle respiratory infections caused by superbugs

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Antibacterial resistance is a global health issue

What you need to know

WHO’s first global report on antimicrobial resistance, with a focus on antibiotic resistance, reveals that it is no longer a prediction for the future. Antibiotic resistance - when bacteria change and antibiotics fail - is happening right now, across the world.
Superbugs, No Drugs!

Over the last 30 years, no major new types of antibiotics have been developed

- Penicillin
- Cephalosporin
- Carbapenem
- Fluoroquinolones

Discovery void
What are bacteriophages?
Phages to combat antibiotic-resistant bacteria

Bacteriophages (phages)
- ‘Bacteria eaters’
- Viruses that can target and kill superbugs
- Replicates inside the bacteria
- Can penetrate biofilm

Inhaled phages therapy:
deliver phages to the lungs to treat bacterial respiratory infections
PEV phages

PEV1, PEV2, PEV10, PEV20, PEV31, PEV40, PEV61
Podoviruses and myoviruses
Effective against *Pseudomonas aeruginosa*

Prof Betty Kutter, Evergreen State College, Washington
Screened against 90 clinical and MDR *P. aeruginosa* isolates collected in Australia
Inhalation aerosol delivery – Liquid Formulation

- Generate inhalable aerosols using nebulisers
- Device: bulky, require electricity, regular disinfection
- Require refrigeration (cold chain storage)
- Phages are under stress during nebulisation (titer loss)
- Suitable nebuliser choice is crucial

https://www.atemwegsliga.de/en-nebulizer.html
Mesh nebuliser

Jet nebuliser

https://www.directhomemedical.com/cart/graphics/00000001/pari-vios-pro-compressor-nebulizer-kit-lc_600x600.jpg
https://www.youtube.com/watch?v=J5GOPTE6bEo
Inhalation aerosol delivery – Powder Formulation

- Inhaler devices: small, portable, not require electricity, better patient adherence

- No cold chain storage required (longer shelf-life, likely cheaper cost for final products)

Osmohaler
(dry powder inhaler)
Production of **inhalable & stable** phage powders

**Spray-drying**

Spray dried powder containing phage and excipients

**Stabilisation**

In vitro biological activity (phage titre)

**Characterisation**

Physicochemical properties (size, crystallinity, thermodynamic properties, dispersibility, phage release)

Stability over time

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Research paper

Production of highly stable spray dried phage formulations for treatment of *Pseudomonas aeruginosa* lung infection

Rachel Y. Chang\(^a\), Jennifer Wong\(^a\), Ash Mathai\(^a\), Sandra Morales\(^b\), Elizabeth Kutter\(^c\), Warwick Britton\(^d\), Jian Li\(^e\), Hak-Kim Chan\(^a,\*\)

• Seven Excipients: lactose, trehalose, mannitol, glycine, leucine, PEG3000, Pluronic F68

Increasing leucine concentration

Increasing sugar concentration

Phage Biological Stability

Powder Physical Stability
In vivo efficacy and safety testing
neutropenic mouse lung infection model

1640 cm$^{-1}$ - 1538 cm$^{-1}$ - 1724 cm$^{-1}$ - 1384 cm$^{-1}$ - 1232 cm$^{-1}$

Khanal et al
Anal Chem
2019 (in press)
a. Microtome the powder

Spray dried phage powder  Embedding in epoxy and curing

AFM-IR measurements

AFM-IR spectra acquired

b. Conventional FTIR

![Image of FTIR scan]

C. NaSt-Lactose  NaSt-lactose-phage

![Graph showing transmittance vs. wavenumber]
Phage and antibiotic combination
Lin et al. 2018 Int J Pharm. 551:158-165

Nebulisation of phage and antibiotic combination
Production of inhalable powders containing phage and antibiotics
In vivo efficacy testing
**Phage and antibiotic combination**
Lin et al. 2018 Int J Pharm. 551:158-165

*In vitro* synergistic efficacy screening

FDA approved five antibiotics for inhalation

Colistin  
Aztreonam  
Tobramycin  
Ciprofloxacin  
Amikacin

1/2 MIC and 1/4 MIC

Phage PEV20

MOI: 0.01, 0.1, 1, 10, 100, 1000

Bacterial killing kinetics study

Bacterial survival rate:

$\text{OD}_{600}$ of treatment group/ negative control
Synergy vs P. aeruginosa FADD1-PA001

Antibacterial activities of phage PEV20 (MOI=0.1) against P. aeruginosa FADD1-PA001 in the presence of 1/4 MIC of ciprofloxacin (CIP), amikacin (AMI), colistin (COL), tobramycin (TOB), and aztreonam (AZT). (n=5)
Antibacterial activities of phage PEV20 (MOI=100) against *P. aeruginosa* JIP865 in the presence of 1/2 MIC of ciprofloxacin (CIP), amikacin (AMI), colistin (COL), tobramycin (TOB), and aztreonam (AZT) (n=5).
No synergy vs *P. aeruginosa* 20844n/m(s)

Antibacterial activities of phage PEV20 (MOI=1000) against *P. aeruginosa* 20844n/m(s) in the presence of 1/2 MIC of ciprofloxacin (CIP), amikacin (AMI), colistin (COL), tobramycin (TOB), and aztreonam (AZT) (n=5).
Phage and antibiotic combination

Lin et al. 2018 Int J Pharm. 551:158-165

Synergy of nebulized phage PEV20 and ciprofloxacin combination against *P. aeruginosa*

Suppress emergence of phage- and antibiotic-resistant bacterial strains
Synergy remains after nebulisation

Calculated and observed bacterial **survival rate** of strain FADD1-PA001 or JIP865 of PEV20-ciprofloxacin combination at 24 h of bacterial growth kinetics study before and after nebulization (n=6).

<table>
<thead>
<tr>
<th>Nebulizer Type</th>
<th>Strain</th>
<th>Calculated</th>
<th>Observed before nebulization</th>
<th>Observed after nebulization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FADD1-PA001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air-jet nebulizer</td>
<td></td>
<td>0.06±0.02</td>
<td>0.02±0.002*</td>
<td>0.03±0.007*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.53±0.07</td>
<td>0.01±0.006*</td>
<td>0.01±0.003*</td>
</tr>
<tr>
<td></td>
<td>Run 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03±0.007*</td>
<td>0.02±0.009*</td>
<td>0.01±0.005*</td>
</tr>
<tr>
<td></td>
<td>Run 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03±0.008*</td>
<td>0.01±0.004*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Run 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrating mesh nebulizer</td>
<td></td>
<td>0.09±0.02</td>
<td>0.01±0.003*</td>
<td>0.05±0.002*</td>
</tr>
<tr>
<td></td>
<td>JIP865</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0±0.3</td>
<td>0.03±0.007*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Run 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01±0.003*</td>
<td>0.01±0.005*</td>
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<tr>
<td></td>
<td>Run 2</td>
<td></td>
<td></td>
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<td>0.01±0.003*</td>
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</tr>
<tr>
<td></td>
<td>Run 3</td>
<td></td>
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</tr>
</tbody>
</table>

*Statistically significant (p<0.05) according to ANOVA.

Note: nebulization was carried out in triplicate using both nebulizers.
Aerosol performance of nebulised phage-ciprofloxacin combinations

Fine particle fraction < 5 µm using the Pari air-jet and eFlow vibrating mesh nebulisers (n=3).

Aerosol particle size distributions (n=3)

<table>
<thead>
<tr>
<th>Nebulisers</th>
<th>Target strain</th>
<th>$D_{10}$ (µm)</th>
<th>$D_{50}$ (µm)</th>
<th>$D_{90}$ (µm)</th>
<th>Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air-jet</td>
<td>FADD1-PA001</td>
<td>1.25 ± 0.16</td>
<td>3.62 ± 0.22</td>
<td>9.82 ± 0.45</td>
<td>2.37 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>JIP865</td>
<td>1.26 ± 0.13</td>
<td>3.72 ± 0.21</td>
<td>9.80 ± 0.45</td>
<td>2.29 ± 0.05</td>
</tr>
<tr>
<td>Vibrating mesh</td>
<td>FADD1-PA001</td>
<td>2.09 ± 0.06</td>
<td>5.12 ± 0.03</td>
<td>12.68 ± 0.04</td>
<td>2.07 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>JIP865</td>
<td>2.11 ± 0.02</td>
<td>5.31 ± 0.09</td>
<td>13.51 ± 0.55</td>
<td>2.14 ± 0.07</td>
</tr>
</tbody>
</table>
Powder Formulations

Components (current dose of ciprofloxacin 32.5 mg)
- **Formulation A**: 35.7% ciprofloxacin HCl, 32.2% lactose, 32.2% leucine, phage PEV20
- **Formulation B**: 68.9% ciprofloxacin HCl, 31.1% leucine, phage PEV20

Spray drying conditions
- Inlet 60 °C
- Aspiration 100% (35m³/h)
- Pump 5% (1.5 mL/min)
- Atomizing airflow 60 mm (742 L/h)
Powder Formulations

Aerosol performance: Fine particle fraction (< 5 µm)
59.7% (A) and 64.3% (B)

Lin et al (2019)
Eur J Pharm Biopharm
142:543
In vitro antimicrobial synergy maintains after aerosolisation

OD 600 at 24h

Ciprofloxacin only  PEV20 only  FADD1-PA001  JIP865

Before  After  Before  After  Negative control

Formulation A aerosolization  Formulation B aerosolization

Lysins – phage lytic enzymes

- bactericidal on contact for rapid killing
- effective vs superbugs, biofilms, and synergistic with antibiotics

Cpl-1, a *Pneumococcal* lysin

Effective against *Streptococcus pneumoniae* in murine models

https://www.rcsb.org/structure/1H09
Endolysin Cpl-1

Before nebulization

Jet nebulizer collected during 14-21 min

Mesh nebulizer

20min

20min

20min

20min
Conclusions

1. We recommend mesh nebulisers over air-jet nebulisers for generating phage and endolysin aerosols

2. We can make inhalable powders with phages remaining biologically intact

3. It is safe and efficacious to deliver phage powders in a murine acute lung infection model with MDR P. aeruginosa.

Where are we headed?
• PK/PD, dose regimen
• Non-neutropenic animal model
• Combination therapy
• Further formulation R&D
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