From modelling to synthesis to formulation to microbe: 
A multi-disciplinary approach to developing treatment for multi-drug resistant respiratory infection

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Antimicrobial resistance (AMR)- a global threat

Increasing incidence of respiratory infection-associated mortality

Antimicrobial resistance (AMR) and respiratory infection

Respiratory infections with increasing AMR incidence include:

- Hospital-acquired pneumonia
  - *Escherichia coli*
  - *Acinetobacter baumanii*

- Cystic fibrosis-associated respiratory infection
  - *Pseudomonas aeruginosa*
  - *Staphylococcus aureus*
What is needed to tackle the problem of MDR-respiratory infection?

1. Discovery of new antimicrobial molecules
   - Desperately need new antimicrobial drugs
   - Should be designed to tackle the cellular mechanisms of MDR
   - Must demonstrate activity against MDR-pathogens

2. Effective way of delivering new compounds to the infected lung
   - Inhalation – enables higher local concentration of drug in the lung
   - Residence time is important – “right drug, right time, right dose”
   - Formulation approaches (e.g. liposomal encapsulation) have been demonstrated to facilitate longer retention and improved clinical outcomes for antimicrobials
Project rationale

• Interdisciplinary approach:

  **Chemistry**
  Computational Synthesis

  **Pharmaceutics**
  Formulation design, preparation and characterisation

  **Microbiology**
  Ability to test drug and formulation against clinically isolated MDR-pathogens

**PROJECT AIM:**

Development, characterisation and biological testing of a liposomal formulation of a novel lead candidate antimicrobial, AB1 which would be suitable for inhalation

*Ready for pre-clinical testing as an inhaled treatment for MDR-respiratory infection*
Methods: Design of AB1 (ARB-drug hybrid)

AB1 – designed by Dr Miraz Rahman AMR Research Group (KCL)

**ARB Fragments criteria:**
- Low molecular weight - typically <150 dalton

**ARB-Antibiotic Hybrids criteria:**
- *In silico* study must show greater affinity and stronger interaction with the binding pocket of the transporter than the unmodified antibiotic
- Must retain the intrinsic mechanism of action of the antibiotic class

Methods: Synthesis of AB1 and the ARB compounds

Example synthetic strategy: ARB derivatives of ciprofloxacin

Synthesise on small scale (20-200 mg)
Test antimicrobial activity

Lead candidate (AB1)
- Improved binding to bacterial target
- Improved antimicrobial activity against MDR-strains
- BUT increased hydrophobicity
- Challenge for inhalation → rapid absorption
Methods: Development of liposomal formulation

Objective 1: Preparation of liposome carrier

Method: Thin lipid film hydration, extrusion and ammonium sulphate gradient loading

Analysis: Size and polydispersity analysed using dynamic light scattering (DLS) (Zetasizer NanoSeries, Malvern Panalytical)
**Results: Preparation of liposomal carrier**

**Outcome 1:** Extrusion produces a good quality liposome suspension with diameter ~150 nm and narrow polydispersity (P.d.I.<0.1)
Methods and Results: Drug loading of liposomal formulation

Objective 2: Load the liposome carrier with AB1 and characterise drug loading

Statistical optimisation:
• Two variables (incubation time, mins, and temperature, °C), 3 levels
• Nine combinations

Analysis: (1) Extract drug using acetonitrile. Quantify using HPLC (2) Size measured using DLS

Results:

Outcome 3: Optimised loading conditions:
• Drug + liposomes at 1:2 ratio
• Incubated at 55°C for 70 mins
Results: Drug loading of liposomal formulation

• **Outcome 4:**
  - Drug loading capacity (%DLC) of 20-25% achieved
  - Comparable to levofloxacin (control)

• **Outcome 5:**
  - Drug loading does not affect liposome size
Methods: Investigation of controlled release properties

Objective 3: Assess the controlled release profile of AB1 from liposomal formulation vs free drug

For fluoroquinilone-type molecules like AB1, rate of drug release across dialysis membrane has been shown to correlate with lung absorption in vivo \(^6\)

**Hypothesis**: Liposomal AB1 will have reduced release rate compared to free drug

**In vitro release assay**:

**Sample**:
- Liposomal AB1
- OR
- Free AB1

**Drug release media**:
150 mM NaCl at 37°C

**Analysis**:
Drug release quantified using HPLC

Samples removed over 24h
Results: Investigation of controlled release properties

- **Outcome 6:** AB1 demonstrated controlled release kinetics from liposomal formulation vs free drug

### AB1 drug release profile

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_{50}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free AB1</td>
<td>4.7</td>
</tr>
<tr>
<td>Liposomal AB1</td>
<td>13.8</td>
</tr>
</tbody>
</table>

### Levofloxacin drug release profile

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_{50}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free levo</td>
<td>5.7</td>
</tr>
<tr>
<td>Liposomal levo</td>
<td>27.5</td>
</tr>
</tbody>
</table>
Methods: Biological testing of antimicrobial activity

- **Objective 4:** Test antimicrobial activity of liposomal AB1 compared to free drug
- Collaboration with Research and Development Institute, National Infections Service, Public Health England
- Antimicrobial susceptibility to AB1 in free and liposomal formulation tested by Minimum Inhibitory Concentration (MIC) testing using the broth microdilution method

- *Bacterial panel used:*

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Species</th>
<th>Antimicrobial susceptibility profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCTC 6571</td>
<td>S. aureus</td>
<td>Susceptible</td>
</tr>
<tr>
<td>NCTC 13616</td>
<td>S. aureus</td>
<td>Resistant</td>
</tr>
<tr>
<td>NCTC 13277</td>
<td>S. aureus</td>
<td>Resistant</td>
</tr>
<tr>
<td>NCTC 775</td>
<td>E. faecalis</td>
<td>Susceptible</td>
</tr>
<tr>
<td>NCTC 12201</td>
<td>E. faecalis</td>
<td>Resistant</td>
</tr>
<tr>
<td>NCTC 12204</td>
<td>E. faecium</td>
<td>Resistant</td>
</tr>
</tbody>
</table>
Results: Biological testing of antimicrobial activity

- **Outcome 7**: Liposomal AB1 retains activity compared to free drug
- Modest improvement (**MIC > 2 fold reduction**) for liposomal AB1 compared to levofloxacin liposomes in *S. aureus* and *E. faecalis* (both associated with MDR-respiratory infection\(^7,8\))

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Species</th>
<th>Antimicrobial susceptibility profile</th>
<th>Free AB1</th>
<th>AB1 liposomes</th>
<th>Free levofloxacin</th>
<th>Levofloxacin liposomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCTC 6571</td>
<td><em>S. aureus</em></td>
<td>Susceptible</td>
<td>0.125</td>
<td>0.03125-0.125</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>NCTC 13616</td>
<td><em>S. aureus</em></td>
<td>Resistant</td>
<td>4</td>
<td>2*</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>NCTC 13277</td>
<td><em>S. aureus</em></td>
<td>Resistant</td>
<td>4</td>
<td>2*</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>NCTC 775</td>
<td><em>E. faecalis</em></td>
<td>Susceptible</td>
<td>0.5</td>
<td>0.25*</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>NCTC 12201</td>
<td><em>E. faecalis</em></td>
<td>Resistant</td>
<td>0.25</td>
<td>0.125*</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NCTC 12204</td>
<td><em>E. faecium</em></td>
<td>Resistant</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
**Future directions:**

**Project output:**
Liposomal formulation of a novel antimicrobial
- Stable ✔
- High Drug loading capacity ✔
- Controlled drug release profile ✔
- Retained/improved antimicrobial efficacy against drug-resistant respiratory pathogens ✔

**Next directions:**

<table>
<thead>
<tr>
<th>In vitro biocompatibility</th>
<th>Formulation development</th>
<th>Pre-clinical testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity and pro-inflammatory potential</td>
<td>Freeze-dried formulation optimisation <em>(in progress)</em></td>
<td>Proof-of-concept study</td>
</tr>
<tr>
<td>Free drug and liposomal formulation</td>
<td>Stability to aerosolization <em>(in progress)</em></td>
<td>Safety and efficacy of liposomal AB1 against <em>S. aureus</em> lung infection in mouse model</td>
</tr>
</tbody>
</table>
Conclusions

- A stable liposomal formulation of a novel antimicrobial lead candidate, AB1, has been developed which shows controlled release properties and retains antimicrobial efficacy.

- The formulation will progress to pre-clinical testing and further formulation development for inhaled use.

- The multi-disciplinary approach combining computational and synthetic chemists, formulation scientists and microbiologists has enabled rapid development of this formulation for use in inhaled AMR-respiratory infection treatment.

- This study has shown the benefits of an interdisciplinary approach to tackle one of the biggest challenges facing global healthcare, antimicrobial resistance, and should serve as a model for greater holistic research approaches to this considerable challenge.
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References


