**Developing New Treatment Options for Infections Caused by Pathogenic Gram Negative Bacteria**

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**Background and aims.** Each year, there are about five million deaths associated with antimicrobial resistance worldwide and this number is expected to increase with the continuing misuse of antimicrobials and the lack of new drugs under development [1]. Carbapenems are a type of ß-lactams which were developed after other antibiotics started losing their efficacy due to resistance. Carbapenem-resistant Gram negative species are now starting to emerge with the WHO listing them in their top priority pathogens requiring immediate attention [2]. There are multiple mechanisms leading to carbapenem-resistance, including intrinsic drug-inactivating enzymes such as the New Delhi metallo-ß-lactamase-1 (NDM-1) [3]. The aim of this study is to resensitise Gram negative bacteria to carbapenems by finding NDM-1 inhibitors through drug-repurposing.

**Methods.** Using the Maestro Schrodinger software, an *in silico* model of NDM-1 was used to screen approved drugs and generally regarded as safe (GRAS) compounds for their binding affinity to the active site of the enzyme. The lead compounds with the best docking scores – labelled resistance breakers (RBs) – were then tested *in vitro* through checkerboard and time-kill assays to assess their synergistic and bactericidal effects with meropenem, ertapenem and imipenem against different resistant strains, such as *Escherichia coli* and *Klebsiella pneumoniae*.

**Results.** *In silico* screening yielded RB1, RB2 and RB3 as lead compounds. The minimum inhibitory concentration (MIC) of each antibacterial agent was first determined against two Gram negative strains which express the gene encoding for NDM-1. The synergistic effects of the RBs were highlighted by their capacity to significantly reduce the MICs of carbapenems in strains expressing NDM-1 (Table 1), but had no activity on those which did not express NDM-1. Further testing also showed the bactericidal activity of carbapenems when combined with the RBs.

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|  | **Antibiotic MIC reduction (folds)** |
|  | ***E. coli* BAA-2452** | ***K. pneumoniae* BAA-2473** |
|  | **Meropenem** | **Imipenem** | **Ertapenem** | **Meropenem** | **Imipenem** | **Ertapenem** |
| **RB1** | 64 | 8 | 64 | 16 | 8 | 64 |
| **RB2** | 8 | 8 | 16 | 4 | 8 | 8 |
| **RB3** | 4 | 8 | 64 | 64 | 8 | 16 |

**Table 1.** The effect of RBs on the MIC of carbapenems in NDM-1 expressing *E. coli* and *K. pneumoniae.*

**Conclusion/Discussion.** So far, the RBs seem to be promising in resensitising Gram negative species to carbapenems. Testing is being extended to include other carbapenem-resistant strains such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* which express NDM-1 and/or other carbapenemases.

**References:**

1. *Fourth australian report on antimicrobial use and resistance in human health*. 2021, Australian Commission on Safety and Quality in Health Care: Sydney.

2. *WHO Bacterial Priority Pathogens List, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance.* 2024, World Health Organization: Geneva.

3. El-Khoury, C., et al., *The role of adjuvants in overcoming antibacterial resistance due to enzymatic drug modification.* RSC Med. Chem., 2022. **13**: p. 1276-1299.