WHITE PAPER

# High-throughput approaches to overcome antimicrobial resistance

## Tackling a global health threat using high-content imaging

### Part One of a two-part series

Antimicrobial resistance (AMR) has been listed by the World Health Organization (WHO) as one of the top 10 global public health threats currently facing humanity.[1] The main driver for AMR is the use of antimicrobials, with misuse and overuse compounding the problem. As a result of drug resistance, antibacterials and other antimicrobial medicines become less effective or even ineffective, and infections become more difficult or impossible to treat, respectively. Despite AMR being recognized as a major global health threat, the current pharmaceutical pipeline for antimicrobials is largely stagnant and unable to tackle the challenge of increasing emergence and spread of AMR bacteria.[2]

To overcome this problem, a better use of existing antimicrobials and new classes of antibacterials are urgently needed, including those that address novel targets and use new modes of action. This process requires a thorough understanding of genetic and phenotypic diversity of clinical collections of bacteria, and the changes that occur when they are perturbed with antimicrobials, antibodies, or other potential interventions.

Prof. Stephen Baker leads a team of researchers at the University of Cambridge that exploit high-content imaging to phenotype the effects of antimicrobial exposure on individual bacteria cells and screen for novel alternatives to existing antimicrobials.

Projects include the development of polyclonal antibody serum, monoclonal antibodies, and functional assays that complement conventional molecular microbiology approaches. Here, in this first part, we explore the use of high-content imaging of *Klebsiella pneumoniae* and the implications for AMR research.

### Novel antibody targets and antimicrobial peptides against *Klebsiella pneumoniae*

*Klebsiella pneumoniae* is a Gram-negative bacterium that can cause a range of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Prof. Baker's group has been using high-content imaging techniques to test antibody binding against a large collection of *Klebsiella* isolates, as well as the potential efficacy of various Antimicrobial Peptides (AMPs).

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The team first used the Opera Phenix® system to characterize the morphological changes of 175 clinical isolates of *Klebsiella* following exposure to key antimicrobial classes. The reason for developing this collection was the realization that the use of traditional typed strains was not wholly representative of the antigenic and phenotypic diversity of the contemporary *Klebsiella* population. Various morphological parameters, including width, length, and roundness were measured and mapped to explore the distribution of phenotypes across the collection of isolates when they interacted with different antimicrobial compounds. Their work recognized that the genetic and phenotypic diversity seen among *Klebsiella* with and without antimicrobial exposure was vast, and represented a paradigm shift in the way that modern bacteriology is conducted.

**Identifying potential antibody targets**

From this collection of organisms, the team selected five informative isolates and generated polyclonal serum using *Klebsiella* Outer-Membrane Vesicles (OMVs) as immunogens. At the time of writing, the group is undertaking the largest high-throughput study conducted in the laboratory to date; they have taken the 175 clinical isolates and tested the five polyclonal serums against them in triplicate and biological repeats (120 96-well plates used in total). They are then imaging the bacterial cells using the Opera Phenix system and subsequently mapping the binding intensities of the different isolates (Figure 1).

The goal of this project is to identify strong/weak binders and assess the binding distribution within the bacterial population. They will analyze various parameters to detect associations with each category.

As the next generation of antimicrobial therapeutics needs to take genetic diversity into account, it is thought the data generated with this approach can be used to inform researchers about dominant/target antigens and antibody targets for future *Klebsiella* vaccines and therapeutics.

## Antimicrobial Peptides (AMPs) against *Klebsiella* species

In a further project, the team are investigating the efficacy of AMPs against *Klebsiella* species. To date, there has been no clinical use of AMPs against *Klebsiella;* however, in other species they have demonstrated increased efficacy, high specificity, and direct attacking properties. The group hypothesized that AMPs could have clinical utility against *Klebsiella,* especially when used in synergy with conventional antimicrobials.

To explore this further, they have been using the Opera Phenix system to image *Klebsiella* treated with AMPs and explore the phenotypic variation in response to these AMPs. They are focusing on a single lead peptide and are testing it against two important clinical *Klebsiella* isolates. The workflow is currently being optimized, but the team hopes this system could be used to screen for further novel compounds in the future.

This is only the beginning of the novel research that Prof. Baker and his team are conducting. Read part two to see how the team is developing Serum Bactericidal Activity (SBA) assays to measure antibody efficacy in higher throughput and how machine learning can be used to predict antimicrobial response.

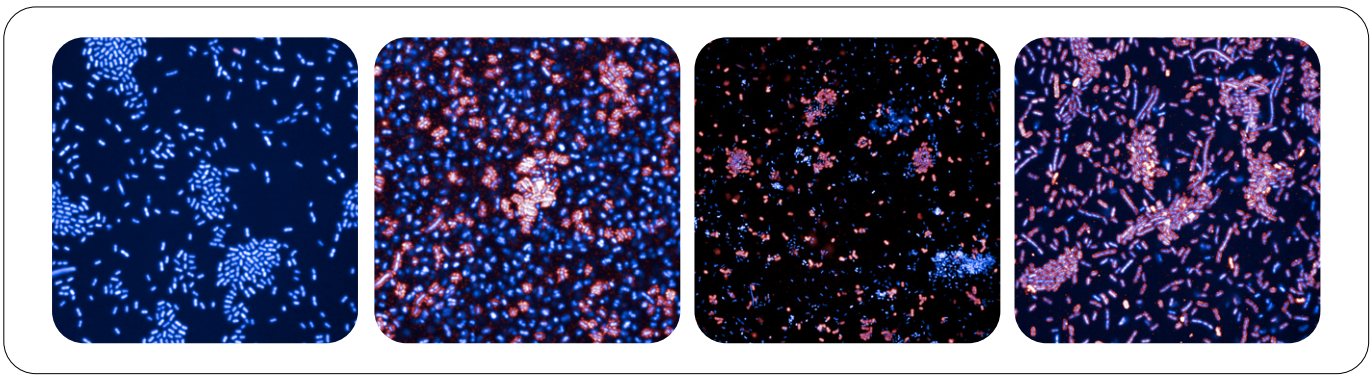

Figure 1: Binding phenotypes of polyclonal serum to *Klebsiella* clinical isolates. *Klebsiella* bacteria in CellCarrier Ultra microplates (Revvity) were incubated with polyclonal mouse serum before being stained with DAPI and anti-mouse IgG AF647 secondary antibody, followed by imaging on the Opera Phenix system in confocal mode using the 63x water objective. The clinical isolates showed a range of binding phenotypes from weak binding (A) to mixed binding (B) to heterologous binding (C) to strong binding (D). Combined with genetic information this work will identify dominant antigens that might be used as target for future *Klebsiella* vaccines or therapeutics.

## References

1. Antimicrobial resistance [Internet]. Who.int. 2022 [cited 16 June 2022]. Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

2. 2020 antibacterial agents in clinical and preclinical development: an overview and analysis [Internet]. Who.int. 2022 [cited 16 June 2022]. Available from: https://www.who.int/publications/i/item/9789240021303

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