WHITE PAPER

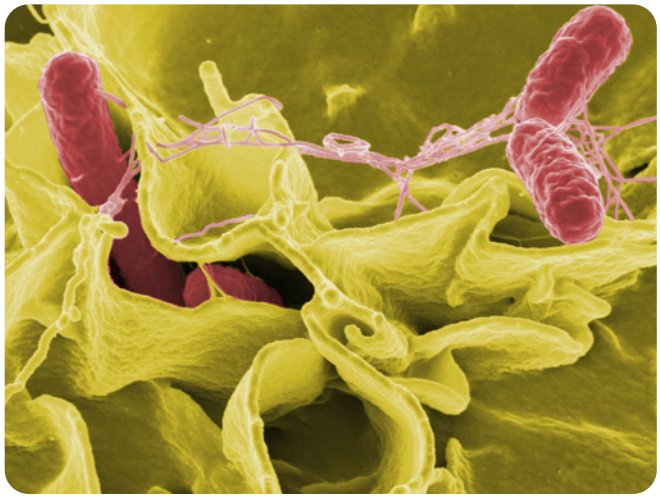
High-throughput approaches to overcome antimicrobial resistance

Tackling a global health threat using high-content imaging

Part Two, of a two-part series

Researchers are having to adopt new ways of thinking and explore alternative therapeutic approaches to tackle the challenge of antimicrobial resistance (AMR). One potential way to curb the problem is through the use of vaccines to reduce the burden of resistant infections. However, for many infections there are no vaccines currently available. This suggests that further work is needed to identify or develop therapeutics or vaccines against bacteria, and to gain a deeper understanding of how bacteria respond to current antimicrobials.

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 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 second part of our research series, we explore the development of assays to measure antibody efficacy in higher throughput and the use of machine learning algorithms to predict how an organism is going to behave in response to an antimicrobial.



Novel high-content assays to measure antibody function and predict antimicrobial resistance

Salmonella Typhimurium is a Gram-negative bacterium that causes diarrheal disease. Most S. Typhimurium cases are mild; however, some patients can develop an invasive disease, which can be life-threatening. As antimicrobial resistant variants have emerged, vaccination is one of the best prophylactic measures to control *Salmonella* infection; however, a licensed vaccine against S. Typhimurium in humans is not yet available. Vaccine development or the development of new antimicrobials against *Salmonella* is therefore very important especially for high-risk groups such as infants, the elderly, and the immunocompromised.

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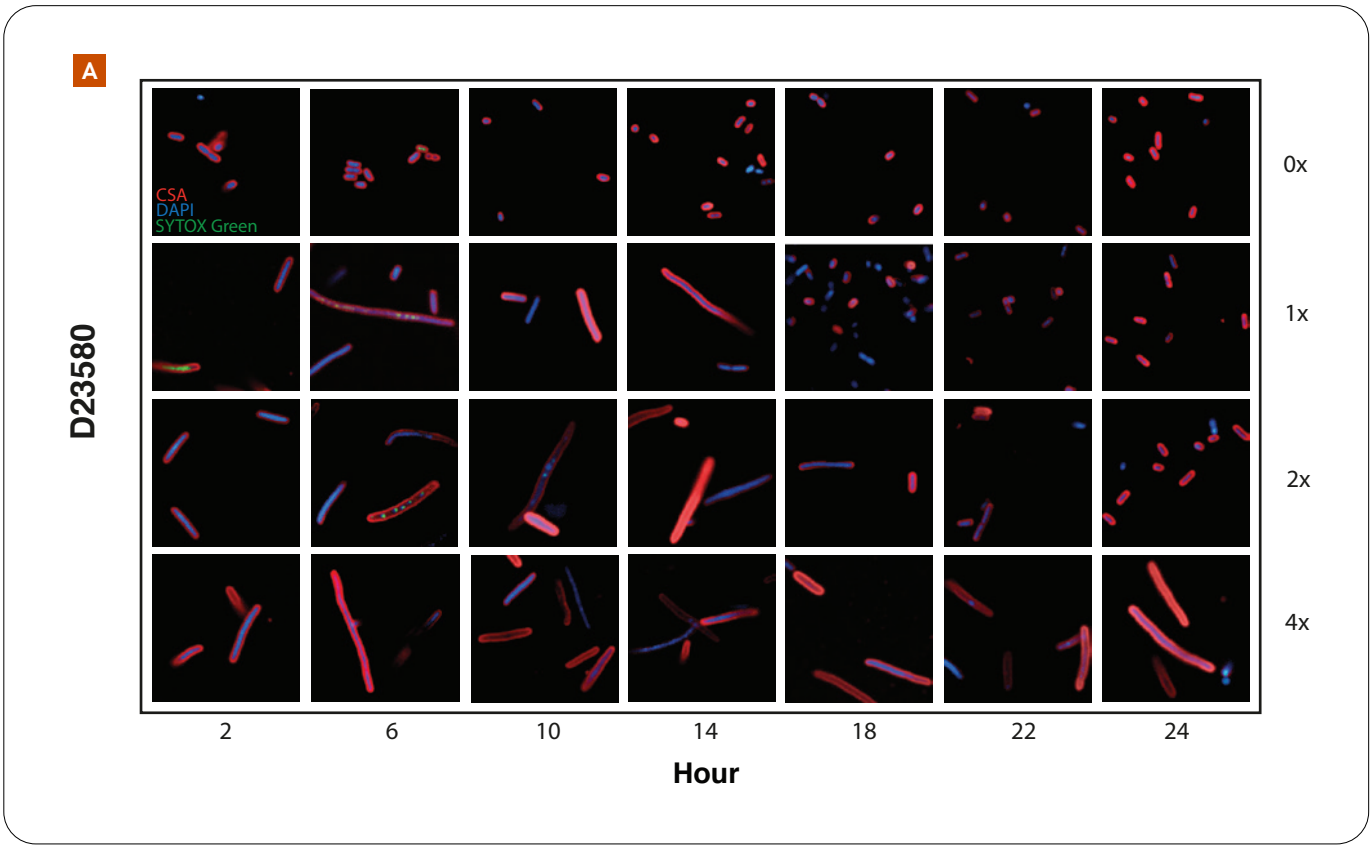

Figure 1: *Salmonella* Typhimurium Strain D23580 morphologic response to Ciprofloxacin. *Salmonella* multidrug resistant strain D23580 was exposed to different concentrations of ciprofloxacin at 0x, 1x, 2x, 4x minimum inhibitory concentration (MIC), labeled with CSA (bacterial cell wall, red), DAPI (DNA, blue) and SYTOX Green (DNA dead bacteria, green) and imaged every 2 hrs on the Opera Phenix® system. Images show huge variation in bacterial length and SYTOX Green fluorescence intensity at different exposure times and MICs.

Serum Bactericidal Activity (SBA) assays measure the complement-mediated killing of bacteria by antibodies in serum and are exploited to evaluate the functional activity of therapeutic antibodies or vaccine-induced antibodies against bacteria. SBA assays are typically performed by incubating a target bacterial isolate with serial dilutions of sera and an exogenous source of active complement. Binding of antibody to the bacterial antigen triggers the classical complement pathway and ultimately leads to the death of the target organism. Serum killing activity is measured by plating the reaction mix onto agar plates and counting the surviving bacterial cells in each serum dilution. A conventional SBA assay is labor-intensive, and not suited to evaluate a large number of serum samples. In addition, the assay lacks a phenotypic readout and cannot quantify the level of bacterial agglutination induced by antibodies.

The Cambridge team is therefore exploring the potential of an equivalent of the SBA, which uses high-content imaging to measure antibody efficacy in higher throughput. To develop this concept, they investigated the role of O-antigen in serum susceptibility and resistance. They conducted an SBA using the Opera Phenix® system with a S. Typhimurium lacking O-antigen, and the equivalent wildtype. They then used Harmony® high-content analysis software to extrapolate how well the serum and bacteria bound (agglutination). This information was then used to determine optimal serum concentrations for future experiments. The researchers think the ability to develop these functional assays and measure killing in high throughput will help reduce error rates and save considerable amounts of time in future vaccine or therapeutic antibody development projects.

Machine learning to understand how bacteria respond to antimicrobials

Another goal of the group is to use machine learning algorithms to predict how an organism will behave in response to an antimicrobial, without ever exposing it to the antimicrobial agent. This approach will not only enhance their understanding of AMR, but also holds potential for diagnosing and detecting drug resistant organisms.

To train the AI model, the researchers exposed different *Salmonella* Typhimurium (strains two resistant and two susceptible) to different concentrations of ciprofloxacin over a 24-hour period. They then acquired images every two hours using the Opera Phenix system and determined various associations between the organisms, exposure time, antimicrobial concentration, and bacteria morphology (Figure 1). The AI model was then used to predict how an organism responds to an antimicrobial and was shown to successfully identify resistant and susceptible isolates without prior knowledge.

Conclusion

In this two-part research series, we have demonstrated different ways that Prof. Baker's team is using high-content imaging to understand the phenotypic diversity of clinical collections of bacteria and how different isolates respond to antimicrobial treatment. They are combining this imaging data with machine learning to predict whether specific clinical isolates are resistant to treatment.

The work being conducted by the team in Cambridge represents a paradigm shift in bacteriology research. Their aim is to continue advancing our understanding of AMR and counter rising levels of resistance through the development of novel vaccines and therapeutics.

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