Project Details	
Project Code	MRCIIAR25Br Van Der Kamp
Title	Overcoming β-lactamase-mediated antibiotic resistance by combining biomolecular simulation and experiment
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Antibiotic resistance threatens human health. β-lactamases cause resistance to β-lactams, the most widely used antibiotics. Class C β-lactamases are not well understood, but variants are emerging that help bacteria evade even 'last resort' treatments. Combining computer simulation and experimental methods can explain how, guiding us to regain the upper hand in the 'biochemical warfare' between humans and bacteria.
Description	Rising antibiotic resistance is a major problem for human health. Resistance to β-lactams, the single most important antibiotic class, usually arises through their breakdown by β-lactamases (BLs). Many BL producing bacteria are multi-drug resistant and may cause untreatable infections. Worryingly, new BL variants conferring resistance are detected frequently. Several BLs that are currently widely distributed world-wide are from the BL classes A, B and D. However, class C BLs are increasingly detected and involved in causing resistance against 'last resort' treatments such as the ceftazidime-avibactam (AviCaz) antibiotic-inhibitor combination therapy. We have previously shown that for BL classes A & D, structural and kinetic data combined with multiscale simulations provides detailed insight into the molecular determinants of resistance conferring activity (e.g. ACS Catal 2020, 2022, 2024; ACS Infect Diseas 2022; JACS 2023). In part due to the relative lack of experimental data, this is a challenge for class C BLs. Therefore, this multidisciplinary project aims to combine simulation, structure determination and enzyme kinetics to understand class C BL-driven resistance against key antibiotic treatments. The proposed project will focus on two key aspects: breakdown of cephalosporin β-lactam antibiotics (BLAs) by class C BLs and the inhibition of class C BLs by diazabicyclooctanone (DBO) and other β-lactamase inhibitors (BLIs). These two together will determine the resistance that BLs will confer against 'last resort' BLA/BLI combination therapies. Throughout, computational and experimental work will be closely integrated. Computational analysis of crucial interactions, catalytic mechanisms, reaction intermediates and conformational behaviour (Van der Kamp, Mulholland) will test hypotheses and help analyse enzyme kinetics (Tooke, Spencer). X-ray crystallography (Tooke, Spencer) will provide the necessary structural data to verify initial hypotheses and allow additional computational modelling. The project will focus on a set of Class C BLs from both chromosomal and plasmid origin where changes in different regions have been shown to increase resistance. Initially, outstanding questions on the detailed mechanism will be addressed. Then, multiscale computational 'assays' will be designed to efficiently predict activity differences (by comparison to existing and new experimental data). This is likely challenging, as exact structures of the variants of interest in complex with the BLAs and BLIs are typically not available. Alongside using recent advances in AI structure prediction (e.g.

	AlphaFold), structures of selected BL-BLA/BLI complexes will be determined experimentally (as these are usually not predicted with sufficient accuracy by AI-based methods). Based on the information gained, we aim to predict new putative resistance-conferring BL variants from computational screening of mutations at key positions, and validate these predictions with experimental determination of beta-lactam hydrolysis and inhibition using steady-state and stopped-flow kinetic methods, along with parallel investigation of antibiotic susceptibility in bacterial killing assays. The project will provide training in cutting-edge techniques in complementary disciplines (computational chemistry, molecular biology/biochemistry) using state-of-the-art facilities in the context of a highly collaborative AMR research environment. It will benefit from Bristol and GW4's excellent access to high-performance computing and X-ray facilities. Mechanistic insights of Class C BL conferred antibiotic resistance can inform both the use of existing antibiotics and the possible development of new beta-lactam antibiotics to evade BL-mediated resistance. To accelerate knowledge transfer, findings will be discussed with our network of local and (inter)national collaborators and presented at conferences prior to publication. We will also exploit the broad interest in antimicrobial resistance through public engagement activities.
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