Background

Synopsis of Academic background. I first graduated from the University of Tasmania in 1987 and worked in the Royal Hobart Hospital microbiology lab before moving to Bristol in 1991 to start my PhD which was awarded in May 1995. I then undertook a serious of post-doctoral positions (Mill Hill, MRC and Southmead hospital) before starting a lectureship at Bristol in 1997. Despite my heavy teaching load, due to my success in research, I was quickly promoted to Senior lecture in 2001 and Reader in 2005. In 2008, I was recruited by Cardiff University and offered the position of Professor of Medical Microbiology and Antimicrobial Resistance. Recently (2020), I was recruited by University of Oxford to head up their Antimicrobial Resistance program and was concurrently, instrumental in the securing of the £100M gift from Ineos to establish the Ineos Oxford Institute on Antimicrobial Research (IOI-AMR) where I have been appointed Director of Biology. In 2020, I was awarded the OBE for my contribution to Microbiology and International Engagement.

**Career Highlights and evidence of Scientific Excellence.**

Pretext

My research can be broadly divided into four main themes: **1.** Mechanisms of resistance. **2.** One-health AMR. **3.** Global epidemiology of AMR. **4**. Neonatal sepsis and **5.** Drug discovery. My research outputs will be discussed under these five main headings and cross-referenced to my 40 selected papers. Where necessary, to evidence impact, I will include additional information such as URLs.

**1. Mechanisms of Resistance.** Since my PhD, I have characterized many novel antibiotic resistance genes including SPM-1, TMB-1, AIM-1, GIM-1 and variances of IMP and VIM. Perhaps the most significant of these discoveries was the discovery and characterisation of NDM-1 (#36, #37 and #38 cited 1256, 3522 and 2727 times, respectively). This discovery rapidly became a global story and headlined the BBC 6 o'clock news on 12th August 2010 following world-wide press coverage – in two days the article generated over 4.7M internet hits, and today the mechanism "NDM" generates 5.95M hits. Examples of media coverage are: https://www.theguardian.com/society/2010/aug/12/the-end-of-antibiotics-health-infections; https://www.dailymail.co.uk/health/article-1302358/NDM-1-Were-blame-indestructible-Indian-

superbug.html; https://www.channel4.com/news/drug-resistant-superbug-threatens-uk-hospitals.

This discovery not only signaled the threat to carbapenems; one of the few classes of antibiotics left, but also highlighted the international trade of "medical tourism" or "added value travel" which was seldom known prior to our work. There was considerable negative reaction in India by the private hospital networks and Indian politicians as our work highlighted the potential plights in international travel and elected surgery. Our discovery also energised the WHO (https://www.wired.com/2010/08/ndm-1-the-world-health-organization-warns-governments/), CDC (https://www.wired.com/2012/06/ndm1-us-hospital/) and the UK government to action the Lord Jim O'Neil reports (https://researchbriefings.files.parliament.uk/documents/CDP-2017-0074/CDP-2017-0074.pdf; https://amr-review.org) and consequently help initiate the Fleming Fund (https://www.flemingfund.org), and the eventually signing of the UN charter on AMR. NDM-1 is one of the few resistant mechanisms afforded its own Wikipedia page (https://en.wikipedia.org/wiki/New_Delhi_metallo-beta-lactamase_1) and our articles studies were forwarded as an REF impact case which was very highly rated (https://impact.ref.ac.uk/casestudies/CaseStudy.aspx?Id=3418) .

The second most noticeable resistance mechanism I was centrally involved in was the discovery of the mobile colistin resistance (MCR) gene (#28 and #32 cited 244 and 3859 times, respectively). Rather like, the discovery of NDM-1, the discovery of MCR elicited considerable global response including statements/articles from UK GOV (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/921039/Ted_Final_version__1318703-v45-One_Health_Report_2019_FINAL-accessible.pdf), FAO (https://agris.fao.org/agris-search/search.do?recordID=US201900436520), and CDC (https://www.cdc.gov/drugresistance/solutions-initiative/stories/gene-reported-mcr.html). Importantly, during the writing of this article, in November 2015, Prof. Wu, Prof. Shen and I met with Chinese Minister of Health (MoH), and Minister of Agriculture (MoA), and spoke to them about the importance of this study and its global political implications and ramifications. Consequently, we successfully persuaded the MoH and MoA to ban colistin throughout all of China. This edict formally came into effect as of July 2016 and was written in Chinese law by April 2017, effectively resulting in over 8,000 tons/year of colistin being withdrawn from animal feed (#31 - cited 164 times). This is one of the most successful and rapid interventions on antibiotic

stewardship and met with significant positive media response (https://www.newton-gcrf.org/impact/stories-of-change/slowing-the-spread-of-antibiotic-resistance/), (https://www.nature.com/articles/d41586-020-02889-y). Subsequent studies have shown that the substantial reduction in colistin use in Chinese farming has significantly reduced the prevalence of MCR positive bacteria and colistin resistance throughout many provinces in China (#6 -cited 48 times). Moreover, in addition to MCR-1, we were the first ones to discover the global wide-spread MCR variant, MRC-3, and its association with successfully dominant plasmids (#25 - cited 429 times). These studies were funded by two MRC awards of which I was the UK PI: DETER-XDRE-CHINA (MR/P007295/1) and DXC-HUB (MR/S013768/1). In addition to co-discovering and naming MCR, we realized that the expression of MCR would have a compromising effect on the bacteria and my group were the first to analyse the physiological balance between glycopeptide resistance mediated by MCR and bacterial fitness (#10 and #24 cited 14 and 88 times, respectively). This work highlighted the effect of MCR expression on outer-membrane topology and its role moderating the immune response. As well as characterising and analysing many AMR genes, I have also lead groups analysing the structure and kinetics of many AMR proteins as typified by the first accurate structure of MCR (#29 – cited 92 times). Similar with NDM, MCR is one of the few AMR mechanisms to have its own Wikipedia page (https://en.wikipedia.org/wiki/MCR-1).

The third significant resistance mechanism I have been involved with was the discovery of the mobile tigecycline resistance genes, tetX which completes the pathway to pan-drug resistance. Our epidemiological surveillance work in humans and animal from China (funded by the MCR grants MR/P007295/1 and MR/S013768/1) discovered the first mobile tigecycline resistance gene and its mechanism of action (hydrolysis) and thereby raising further evidence on our global journey to pan-antibiotic resistance (#11 – cited 202 times). We are now the leading group on tigecycline resistance, and similar to MCR, have set up the nomenclature for naming tigecycline resistance genes. My recent studies in Pakistan have, worryingly, identified *tetX5*, *mcr-3* and $bla_{NDM}$ genes carried on the same plasmid.

In addition to discovering two of the most important antibiotic resistance genes, my work has naturally navigated to analysing their mobile genetic elements. Accordingly, I have discovered, characterized and name many novel plasmids, transposons, ICE and integrons. Most importantly, (together Mark Toleman), I was the first to name, characterise and analyse the rolling circle

transposition mechanism of ISCR elements which can mobilise large sections of DNA (>20kb) and have shown to be transferable between bacteria. Consequently, ISCR elements are now regarded as a major mechanism for mobilising antibiotic resistance genes between bacterial plasmids and chromosomes (#39 - cited 678 times) and can be highly plastic via homologous recombination involving class 1 integrons (#40 - cited 143 times).

2. **One-health AMR.** Since our discovery of NDM-1 and my follow up article examining the widespread distribution of NDM in the Indian environment (#36 cited 1256 times) caused considerable attention on the role of poor sanitation in South Asia and how this might exacerbate the dissemination of AMR globally (https://www.nature.com/articles/news.2011.218), (https://www.sciencedaily.com/releases/2011/04/110406214332.htm), (https://www.theguardian.com/world/2011/apr/07/superbug-gene-rife-delhi-water), (https://www.bbc.co.uk/news/uk-wales-south-east-wales-12989782). The full story of NDM and its implication to public health across South Asia was described in our commentary ("The new medical challenge: why NDM-1? Why Indian?"; doi: 10.1586/eri.10.159). This study provided, for the first time, a definitive link between sanitation, environmental contamination, and the dissemination of antimicrobial resistance. Moreover, straight after the publication our article, the Indian government (unhelpfully) responded by issuing chloride tablets. Since our article, the Indian government still have considerable issues with the cost of the lack of sanitation to the Indian communities (https://www.reuters.com/article/us-india-sanitation-idUSTRE6BJ4AP20101220). In 2015, the role of the environment in disseminating AMR was still embryonic and I was approached by the World Health Assembly to co-write an article (with Antoine Andremont) highlighting the importance of what we now consider to be "One-Health" ("The role of sanitation in the development and spread of antimicrobial resistance", AMR Control 2015, Overcoming Global Antimicrobial Resistance). The term "one-health" elicits many different opinions, so I felt that it was necessary to give this term a more concise scientific definition with a commentary in *Nature Microbiology* (#20 – cited 36 times).

In 2014, I formally started working with Yang Wang (promoted to Professor in 2018 on the back of our collaborative work) at the China Agriculture University (CAU), originally on the rapid spread on NDM-positive bacteria throughout Chinese farms which resulted in us understanding the unique and disturbing properties of plasmids such as IncX3 (#15 and #27 – cited 39 and 256 times respectively). During July 2015, Yang shared some preliminary data on a mechanism of

mobile colistin resistance (MCR as described above), and as the *Escherichia coli* were first isolated from chickens, this further raised the issue of interactions between human, animal and environmental (#19 and #28 -cited 80 and 244 times, respectively). Understanding the complexity of the problem, the studies we initiated and undertook in China (as joint collaborations funded by MRC MR/P007295/1) and MR/S013768/1), were, and still are the largest "One health" studies. Trying to understand the pathways to MCR (and NDM) dissemination, we sampled chickens (#6 – cited 62 times), pigs (#6 – cited 62 times), household pets (#9 – cited 17 times), fisheries (#14 – cited 24 times), human normal flora (#15 and #28 cited 39 and 244 times, respectively) and human infections (#21, #26 and #28 cited 35, 40 and 244 times, respectively). Each study also examined MCR (and NDM) negative isolates so as not to bias the study. These vast epidemiological studies also provide a reference point for examining the effect of interventions e.g. the impact of removing colistin as an animal feed (#6 – cited 48 times). Concurrent with these studies, P I collaborated with the Madlen Davies at the Bureau of Investigation Journalism (https://www.thebureauinvestigates.com/profile/madlendavies) to write an article on the use of colistin on Indian farms (#22 – cited 33 times) which was positively addressed by the Indian Ministry of Agriculture (https://www.thebureauinvestigates.com/stories/2019-07-22/india-bans-use-of-last-hope-antibiotic-colistin-on-farms). In addition to my studies in China, I have also studied a "One health" approach using genomic sequencing also showed, for the first time, clonal carriage of *Klebsiella pneumoniae* in flies (#13 – cited 14 times) and more recently the connectivity between surgical site infections and arthropods in Northern Pakistan (#2).

**3. Global epidemiology of AMR.** In addition to my "one-health" work in China, I have led on many national and international clinical surveillance projects examining the spread and impact of AMR. These include: **A**) The first multi-centre study on the clonal spread of carbapenem resistant (VIM-2) *Pseudomonas aeruginosa* (#35 – cited 190 times), **B**) A global assessment of the spread of MDR *Escherichia coli* clones (#34 – cited 469 times), **C**) European survey on carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* (#30 – cited 374 times), **D**) The first study examining the global burden of AMR in low-middle income countries (LMICs) (#12 – cited 75 times), **E**) The first anthropological and socioeconomic study on global antimicrobial resistance (#18 – cited 273 times), **F**) An outbreak of carbapenemase 2-producing *Klebsiella pneumoniae* sequence type 16 in Sao Paulo, Brazil (#8 – cited 7 times), and finally, **G**) A unique review examining the logistics and practicalities of bacteriology in LMICs (#23 – cited 94 times).

4. **Neonatal sepsis**. Due to my work in low-middle countries (LMICs), I often visited hospitals and became particularly interested in neonatal sepsis and mortality. In 2014, the Gates Foundation approached me to establish an international LMIC program on AMR and neonatal sepsis which I termed BARNARDS (https://barnardsgroup.wpcomstaging.com). The program enrolled over 36,000 mothers and over 38,000 newborns and is the largest prospective study in the world examining neonatal sepsis and mortality. Thus far, we have published two large studies; firstly, the largest genomic study on neonatal sepsis (#4 – cited 19 times since April 2021) and the first study to examine the efficacy of dosing relating to cost and average household income (#3 – published in mid-August 2021). Moreover, using the BARNARDS cohort, we have undertaken the largest study on maternal and neonatal normal flora examining the carriage of carbapenem resistance genes (not cited as top 40 but under advance review in *Nature Microbiology*). BARNARDS seamlessly also combines AMR surveillance and aspects of "one-health" as we earnestly sampled maternity wards, NICUs, incubators, resuscitaires etc. and showed connectivity between the hospital environment and isolates causing neonatal sepsis involved in outbreaks or epidemiological clustering (as yet unpublished). In addition to BARNARDS, we have undertaken the most in-depth analysis of neonatal sepsis in South Asian and high-lighted the significance of an outbreak and its acquisition of an AMR plasmid aligned with therapy (#17 – cited 20 times).

5. **Discovery and Evaluation of novel antibiotics and anti-resistance drugs.** During the course of my career, I have used our strain collection and expertise in antimicrobial testing to evaluate new and derivatives of tetracyclines, polymixins, ß-lactams/ ß-lactamase inhibitors and aminoglycosides. I have also explored combinations of existing antibiotics examining synergistic effects including developing methods to accurately assess synergy and antagonistic effects. This approach is exemplified by our in-depth and in-vitro and in-vivo analysis of colistin and partnering compounds to over-come colistin resistance, mediated by MCR (#16 – cited 7 times). One novel molecule we helped develop was the beta-lactamase inhibitor, aspergillomarasmine, a novel structure and has been modifed for further drug development to treat life-threatening Gram-negative infections (#33 – cited 407 times). We have also discovered and developed novel compounds such as small peptides (e.g. RTA-4) up to phase 1 clinical trials. Such is our expertise, that we are one of the leading microbiology laboratories in the ENABLE program (https://www.imi.europa.eu/projects-results/project-factsheets/enable) and have been

fundamental in working with Oxford Chemistry to develop and refine their indolcarboxylate metallo-9-lactamase inhibitors as a candidate molecule (termed 2915) partnering meropenem for serious Gram-negative infections (#1 – in press [Nature Chemistry]). The combination of 2915 and meropenem has been adopted by the IOI and will progress to phase 1 clinical trials.