

Exploring zoonotic pathogens in Australian livestock

Antimicrobial resistance is a growing issue preventing the effective treatment of numerous parasitic diseases in both humans and animals. The lack of effective antimicrobials for the treatment of a growing number of diseases represents a serious threat to global public health, making it imperative that governments and societies around the world take action to tackle this issue. Scientists at the University of Murdoch in Australia are studying antimicrobial resistance in livestock – which can potentially spread to humans – using a statistical approach of surveillance to monitor the situation more closely.

Antimicrobial resistance (AMR) is causing a growing number of standard antibiotic, antifungal, antiviral and antimalarial treatments to become ineffective, leading to increased prevalence and spread of many infectious diseases. This issue is not isolated to human medicine, and is a growing concern for veterinary medicine. It is particularly problematic in livestock, due to a combination of relatively concentrated animal populations and the rise of drug-resistant zoonotic pathogens that can spread between animals and humans.

Dr Sam Abraham, a microbiologist at Murdoch University in Perth, Australia, is investigating AMR at the interface between human and animal health, primarily by studying zoonotic

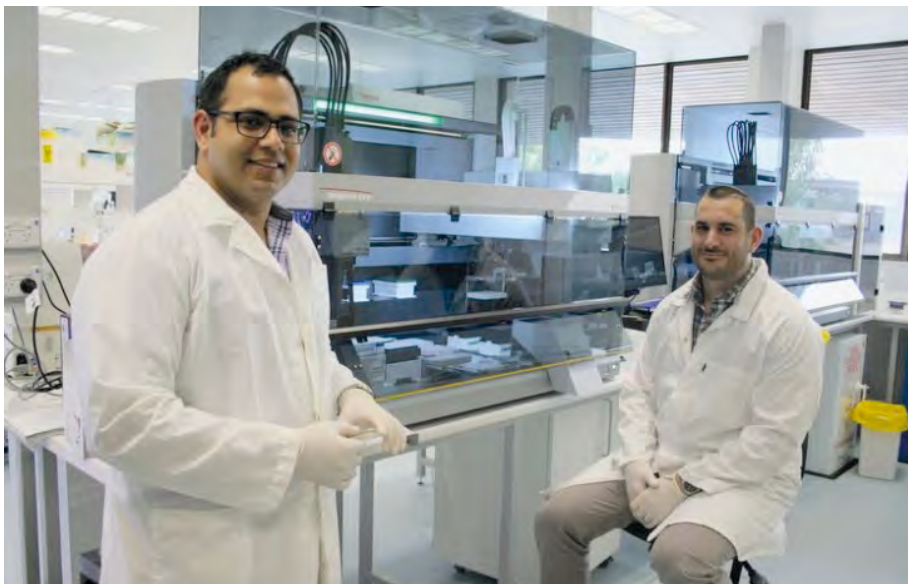
pathogens that are present in Australian livestock. Sam explained: “Antimicrobial-resistant bacteria that emerge and spread in livestock and other animals contribute to the global burden of AMR. As a result, there is a significant public health concern over the transfer of such bacteria to humans via food and the environment, and the rapid transmission of the genes responsible for AMR into other human pathogens.”

“Our lab focuses on determining the minimum inhibitory concentration (MIC) of drugs for bacteria found in fecal samples from chickens, pigs, sheep and cattle – such as *E. coli*, *Salmonella*, *Staphylococcus* and *Streptococcus* – and characterizing the genomic mechanisms of drug resistance in these bacteria. This

project is primarily funded by the Australian government, but we also have global collaborators. The aim of this work is to create a snapshot database of populations of enteric bacteria and, once optimized, use it to monitor levels of antibiotic resistance in livestock all over the world.¹ We use a range of techniques, including classical antibiotic susceptibility testing (AST), RT-PCR, NGS, MALDI-TOF and traditional bacteriology methodologies, such as gram staining.”

“This type of surveillance project requires large-scale sampling of indicator organisms to identify AMR, and monitor whether these resistances are being detected more frequently,” Sam continued. “Unfortunately, most countries only use low numbers of animal samples for surveillance, which is unlikely to highlight emerging issues until it is too late. This is because conventional, human-centric surveillance methods are hamstrung by high processing costs and slow turnaround times, making them incompatible with the high volumes of sampling required to accurately depict a population’s AMR status. We therefore need to use high throughput automation and genomics solutions with rapid turnaround times, enabling the analysis of large data sets to help identify emerging problems quickly, and respond to those problems rapidly.”

“I was initially introduced to Tecan when I was a postdoc setting up the first national network on AMR



Tecan's automated solutions and library preparation kits are helping Sam Abraham (left) and his colleagues to study antimicrobial resistance in livestock

surveillance of Australian animals. I saw one of its trade displays for microbiology and chatted to the very knowledgeable team, who helped us to select a solution that would best suit our AMR screening requirements. In 2016, we were able to purchase our Tecan systems using grants from the Department of Agriculture and Australian Pork Limited. The Tecan team here in Australia was pivotal to this project, partnering with us on the grant application, and working closely with us to design an automation set-up that would meet our needs. We currently have two Freedom EVO® 150 workstations, one of which has a number of SciRobotics devices integrated onto it to create our Robotic Antimicrobial Susceptibility Platform (RASP).²

“Our workflow starts with a homogenized sample, which is transferred to the Freedom EVO – where it could potentially undergo various dilutions depending on the sample – and automatically plated out using the PetriPlater™. The plates are stacked and stored in a PetriSel™, and all of our tubes and plates are barcoded so that the samples can be automatically tracked. This makes the whole system very efficient, and troubleshooting is much easier if there are ever any problems. We plate on chromogenic agar, which allows our PickoloMI™ robotic colony picker – programmed with assistance from Tecan – to easily identify and differentiate specific micro-organisms.

“The number of errors that we generate is also hugely minimized – the beauty of automation – and this results in excellent quality data.”

These colonies are then picked and grown – either for a few hours or overnight – in a 96-well plate.”

“From here, there are different routes of analysis depending on the sample and what we are looking for. Some we send to MALDI-TOF mass spectrometry analysis for bacterial identification, or we perform AST in house to determine the AMR profile. We have also recently integrated next generation sequencing with our microbiology workflow, using a Freedom EVO 150 to perform automated library preparation with Tecan’s Celero™ EZ DNA-Seq library preparation kit. This allows us to rapidly perform genotyping or whole genome sequencing on bacteria that have demonstrated AMR. Genotyping gives us the full picture about the resistant pathogen: is it human-, livestock- or even gull-derived? Is it only a problem for animal health, human health, or both? We can answer all of these questions with genomics. All of the data generated is then sent to and stored in our national database.”

“Using automation, we can process 5,000 to 10,000 bacterial isolates a week – around 150 plates a day – compared to a couple of hundred manually. This means that we only need

one researcher working on this at a time and, as our throughput has increased so significantly, others can spend time on different tasks, which is an incredibly efficient time saver. The number of errors that we generate is also hugely minimized – the beauty of automation – and this results in excellent quality data, which is fantastic. We are very happy with the instruments,” Sam concluded.

1) Laird TJ, *et al. J Antimicrob Chemother*, 2021. doi: 10.1093/jac/dkab403. Online ahead of print.

2) Truswell A, *et al. J Antimicrob Chemother*, 2021, **76**, 1800-1807. doi: 10.1093/jac/dkab107.

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To find out more about Tecan and SciRobotics’ colony picking and plating solutions, visit

www.tecan.com/colony picking

To learn more about the School of Veterinary and Life Sciences at Murdoch University, go to
www.murdoch.edu.au/school-of-veterinary-and-life-sciences/