

HIGH THROUGHPUT AUTOMATION AND GENOMICS FOR AN AMR SURVEILLANCE SYSTEM.

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Antimicrobial resistance (AMR) is a growing global health issue, preventing the effective treatment of numerous diseases in humans and animals. Current AMR surveillance methods in animals are inefficient and ineffective, further complicating the issue. However, automated liquid handling systems and high throughput sequencing platforms mean that it is now possible to identify AMR in large numbers of samples obtained from food-producing animals, helping to stop drug-resistant genes from entering human populations.



AMR impact

Globally, AMR is on the rise and is a challenge arguably as pressing as climate change. It has emerged through inappropriate use of antibiotics, the use of antibiotics without prescription and, more importantly, a lack of new medications in the pipeline to treat bacterial infections. The issue isn't isolated to human care, as many of the same or closely related antibiotics are also used to treat animals. This can result in drug-resistant pathogens evolving in animals, then being transferred to humans – and carrying the resistance genes – via the food chain. The increasing number of drug-resistant pathogens found in animals can lead to:

- Increased human morbidity
- Increased human mortality
- Reduced efficacy of related antibiotics used in humans
- Increased human healthcare costs
- Increased potential for carriage and dissemination
- Faster emergence of resistance in human pathogens

As a result, AMR acquired from food-producing animals is an imminent threat to human health and must be detected before being transferred to humans, which is where AMR surveillance steps in.

AMR surveillance

AMR surveillance systems are used specifically to look for resistant pathogens that emerge in food-producing animals – providing a vital barrier to safeguard human health. Even though these systems continually operate on a global level, current practices are inadequate. The main issue is cost, as just one isolate costs around AU\$300 from start to finish, equating to AU\$60,000 per year for one bacterial species from one source. This leads to low sample sizes and, ultimately, limited conclusions. Denmark, for example, has one of the best surveillance systems in the world on pig farms. Still, on average, only 193 colonies of *E. coli* are investigated each year. This is hardly an accurate representation and certainly not enough data to guide critical AMR decision-making. Laboratory automation offers a potential solution to this problematic scenario, allowing large numbers of samples to be cost-effectively processed to deliver more tangible evidence to help preserve these life-saving medications.

Robotic Antimicrobial Susceptibility Platform (RASP)

RASP is an automated platform designed for high throughput AMR surveillance for various applications, including food-producing animals. It can provide rapid and robust data to advise veterinarians, farmers and government bodies on AMR in animal populations. Because AMR changes with time and location, this flexible platform is capable of capturing both existing and emerging resistance and can potentially provide insights into evolving patterns.

Crucially, the platform can process and examine the huge quantities of samples required to provide robust data. By combining laboratory automation from Tecan®, MALDI-TOF from Bruker® and Illumina® sequencing into a single high throughput solution, it is possible to rapidly screen thousands of samples and study AMR in a large number of colonies, while adhering to internationally recognised standards.



RASP method

1. A sample is taken from the field – in this case, from food-producing animals.
2. The sample is automatically prepared and spiral plated onto selective agars using technologies from SciRobotics® and Tecan to generate varying concentrations.
3. Automated colony picking – which can basically harvest the entire plate – is then used to isolate individual colonies into separate wells of a 96- or 384-well plate, where they can be frozen before subsequent testing.
4. Testing options include:
 - MALDI-TOF for bacterial identification.
 - MIC susceptibility testing, using a 96-well plate according to the Clinical and Laboratory Standards Institute guidelines.
 - Whole-genome sequencing using next generation sequencing.

The primary problem with traditional surveillance is that it picks just one random isolate that is not representative of AMR in that species. With RASP, the entire process is automated, making it possible to process thousands of isolates to generate robust, representative AMR data. In just two weeks, up to 4,000 isolates can be processed at a fraction of the cost of traditional methods – just AU\$6 per isolate for MIC susceptibility testing.

These data can then be used to identify highly resistant strains and the carriage of resistance within a species and between farms. The data can also be sorted into a national database, and more importantly, communicated back to farmers, veterinarians and policymakers to guide appropriate changes in treatment.

Combining automation and genomics to make an impact

Methicillin-resistant *S. aureus* (MRSA) causes significant health issues in humans and animals. In Australia, a highly virulent strain of MRSA was identified that jumped from humans to pigs, then subsequently re-infected humans. Using high throughput automation and genomics, it was possible to track the origin of this strain. It was found that various human MRSA strains colonised Australian pigs through farm workers from Australia, China and Europe.¹ This information led to increased farmer education and procedural changes, where

workers started wearing masks and using improved hand hygiene.

Another study discovered that some seagulls carry drug-resistant strains of bacteria from humans. Again, genomics and automation were used to conclude that seagulls were acquiring *E. coli* strains from humans that are highly resistant to last-line antimicrobials.² In both these cases, this technology helped to provide a comprehensive understanding of how AMR in animals is linked to human health.

Conclusion

Combining automation and genomics is the future of microbiology. Upscaling using advanced liquid handling platforms allows us to rapidly evaluate AMR interventions, implement changes in bacterial management strategies, investigate outbreaks and screen pathogens in both humans and animals. RASP is a next generation approach in AMR surveillance that significantly increases throughput and reduces cost, leading to better decisions and policymaking to reduce the global impact of AMR.

Literature

1. Sahibzada, S., Abraham, S., Coombs, G.W. et al. Transmission of highly virulent community-associated MRSA ST93 and livestock-associated MRSA ST398 between humans and pigs in Australia. *Sci Rep* 7, 5273 (2017). [www.doi.org/10.1038/s41598-017-04789-0](https://doi.org/10.1038/s41598-017-04789-0)
2. Mukerji, S., Stegger, M., Truswell, A.V., et al. Resistance to critically important antimicrobials in Australian silver gulls (*Chroicocephalus novaehollandiae*) and evidence of anthropogenic origins, *Journal of Antimicrobial Chemotherapy*, Volume 74, Issue 9, September 2019, Pages 2566–2574, [www.doi.org/10.1093/jac/dkz242](https://doi.org/10.1093/jac/dkz242)

Additional resources

To explore Tecan's veterinary solutions, visit: www.tecan.com/veterinary

To find out more about Tecan and SciRobotics' colony picking and plating solutions, visit www.tecan.com/colonypicking

To learn more about the Antimicrobial Resistance and Infectious Diseases Laboratory (AMRID) at Murdoch University, go to <https://amrid.com.au/>



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