DOGSTAILS: Rapid diagnosis of bacterial infections using DNA sequencing

Natalie Ring, Alison Low, Bryan Wee, Gavin Paterson, Tim Nuttall, Richard Mellanby, David Gally & Ross Fitzgerald

The Roslin Institute & Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK

ANTIMICROBIAL RESISTANCE IS A GLOBAL HEALTH ISSUE

Antimicrobial resistance (AMR) happens when microbes, e.g. bacteria, develop mechanisms to defend themselves against the medicines (antimicrobials/antibiotics) we use to treat them.

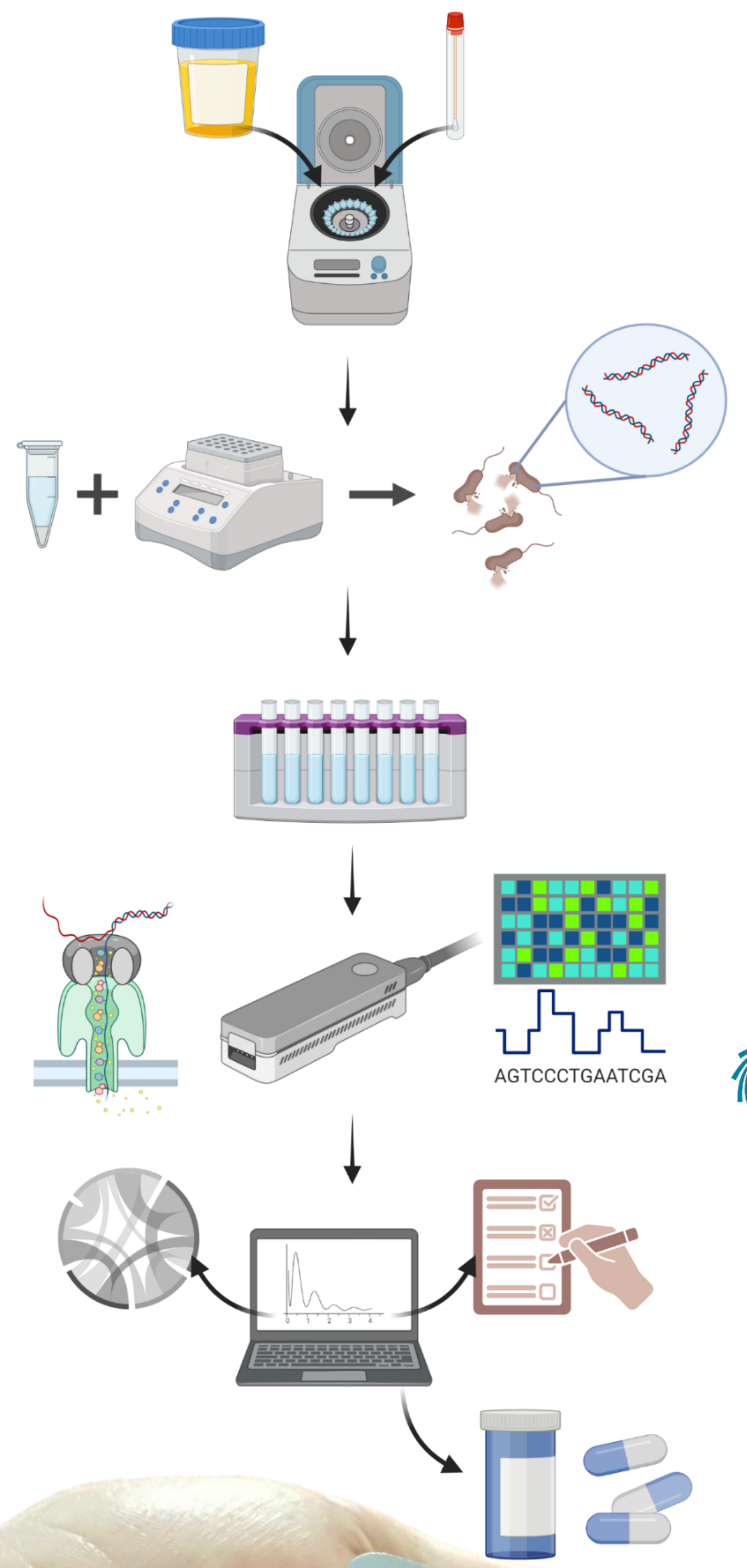
- The more we use antimicrobials, the more **opportunity** we are giving microbes to **develop resistance** to them
- **Levels of AMR are increasing**, driven by **excessive or inappropriate** use of antimicrobials
- We are now seeing some infections, e.g. Tuberculosis, become **resistant to most, or all, known antimicrobials**[1]

The World Health Organisation classifies AMR as one of the biggest threats to global health, food security and development[2]. In 2019, 3.57 million human deaths were related to AMR[3].

We can combat AMR by using antimicrobials responsibly. This means using the right medicine, for the right infection, at the right time.

THE DOGSTAILS PIPELINE

Our new diagnostics pipeline uses nanopore DNA sequencing to identify the bacterial species and potential AMR present in clinical canine urine and skin swab samples, in a fraction of the time taken by the current methods.



Phase 1
- Collect sample from patient with suspected infection

Phase 2
- Centrifuge at max speed for 20 minutes

Phase 3
- Add lysis enzymes
- Incubate for 90 minutes
- Break down cell walls
- Release DNA

Phase 4
- Suspend DNA on magnetic beads
- Pellet on a magnetic rack
- Wash away contaminants

Phase 5
- Sequence DNA for up to 2 hours on nanopore device
- Real-time access to results

Phase 6
- Analyse DNA as it is being sequenced
- Compare to databases of known species and AMR genes
- Diagnose the infection
- Select an appropriate antimicrobial treatment!

Total time: 5-8 hours

ACTUAL SIZE:

MinION (min-eye-on) DNA sequencer from Oxford Nanopore Technologies

WHAT'S NEXT?

- Continue to optimise the protocol for **urine and swab samples**, focussing on AMR prediction in samples with **high levels of host DNA**
- Test other frequently seen clinical sample types, including **blood, tissue, faeces, and various effusions**, and samples from **other animals**
- Open a new lab on-site at the R(D)SVS Hospital for Small Animals, where we will offer the Dogstails method as standard

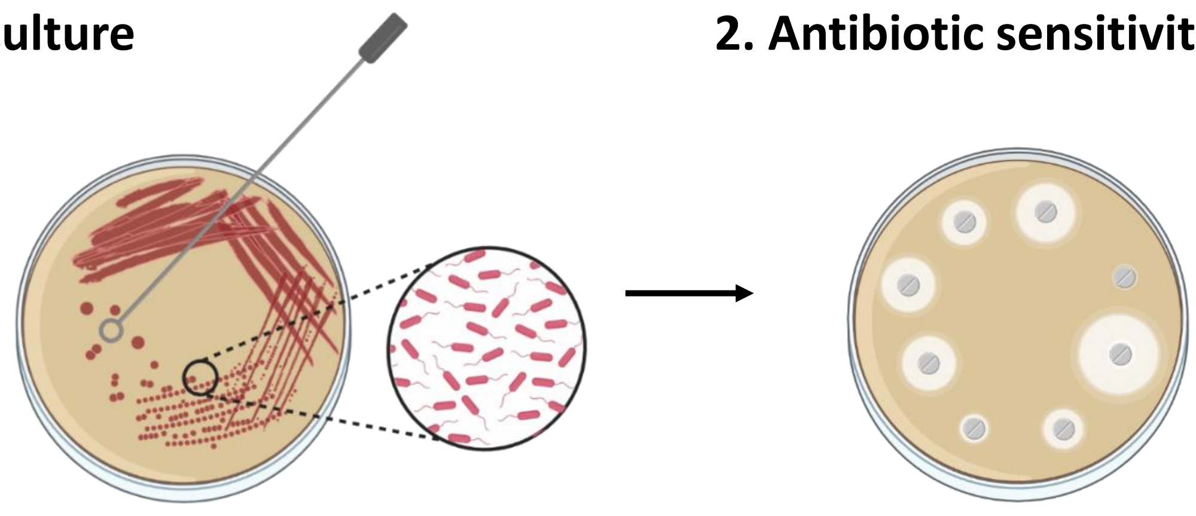
We will continue to demonstrate that a single, adaptable, sequencing-based diagnostic method can enable the rapid selection of appropriate antimicrobial treatments.

CURRENT DIAGNOSTIC METHODS

The current "gold-standard" diagnostic pathway is similar for all sample types. **While waiting for results, broad-spectrum antimicrobials are usually started.**

1. Culture

2. Antibiotic sensitivity testing



Samples are spread on a growth medium, and the resulting bacterial colonies are inspected.

Bacteria are grown in the presence of an array of antibiotics, to determine which they are resistant/susceptible to.

Minimum time: 48+ hours

HOW WELL DOES IT WORK?

We can detect the top ten species most commonly seen in urine and skin infections

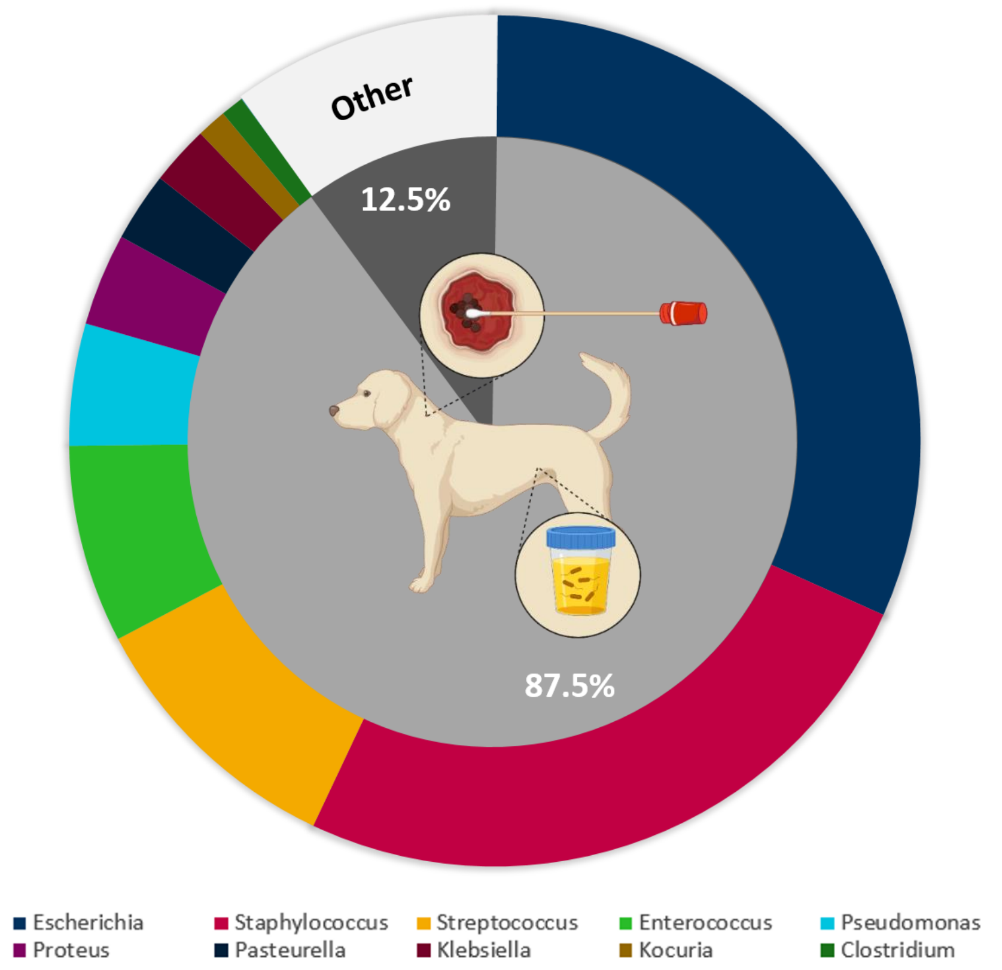


Figure 1: Bacteria diagnosed in HfSA urine and skin infections, 2018-19. 87.5% of cases were diagnosed with one of just ten species

- We optimised the protocol using the ten most common species, representing **87.5% of diagnoses**
- We have subsequently processed **over 50 real clinical samples** with the Dogstails pipeline
- Our lower limit of detection is **comparable to current methods**
- Above this limit, we can identify the **correct species** with **100% sensitivity and specificity**

- **Levels of dog DNA** in the original samples affect the accuracy of AMR prediction
- **Urine** contains fewer dogs cells, and allows **up to 96% accuracy**
- **Skin swabs** contain almost entirely dog DNA, and will **require an extra dog cell depletion step** prior to DNA extraction to enable equally accurate AMR prediction

REFERENCES

1) Seung, K. et al. (2015). Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Cold Spring Harb Perspect Med, **5**(9)
2) WHO (2020). Antibiotic resistance 2020. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance
3) AMR Collaborators (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet, **399**(10325)

"Steph the *Staph*" was created by Eliza Wolfson for the comic *Host Busters!* in collaboration with Joana Alves and the LBEP team at the Roslin Institute. See the whole comic: https://lizawolfson.co.uk/portfolio-item/host-busters-2/

This work is available as a preprint on bioRXiv:

Many thanks to Oxford Nanopore Technologies for sponsoring my travel to STEM for BRITAIN 2023

ABOUT THE AUTHOR

I am a Postdoctoral Research Fellow at the Roslin Institute and Royal (Dick) School of Veterinary Studies in Edinburgh, where I live with my husband and our toddler, and am originally from Essex. I am developing ways of applying DNA sequencing to improve animal welfare.

Some figures created with BioRender.com