Developing dairy-specific molecular screening tools to quantify the within-herd dynamics of antimicrobial resistance

Jolinda Pollock

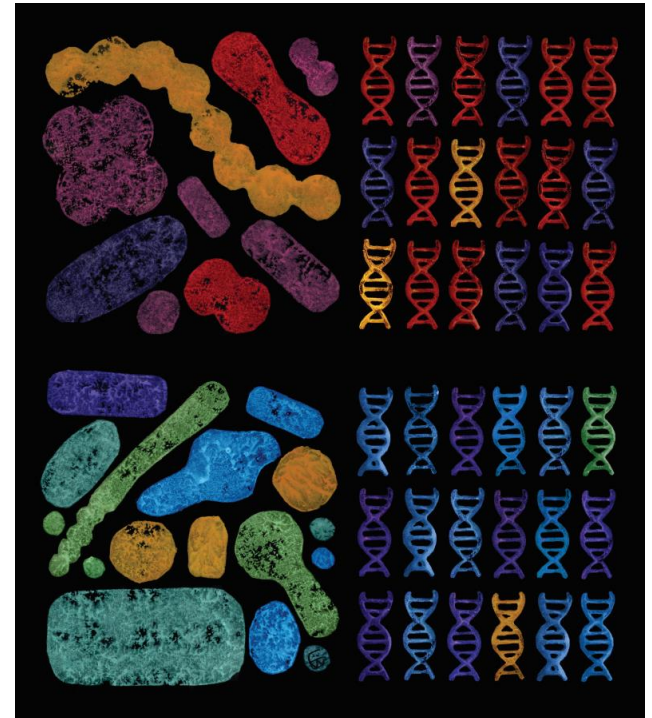**Postdoctoral Research Fellow**
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#milkbugproject

Presentation outline

- Background

- Experimental design

- Tools for measuring AMR:

  – Antimicrobial sensitivity tests

  – AMR gene quantification

  – Whole genome sequencing

- Current results

- Ongoing work

By Eliza Wolfson - @eliza_coli

Background

- There are increasing concerns regarding the overuse of antibiotics due to **antimicrobial resistance** (**AMR**)

- Historically, on dairy farms, all cows were administered antibiotics during dry-off to prevent/treat mastitis

- Due to AMR, farmers now encouraged to consider administering antibiotics to cows at risk of contracting mastitis – selective dry cow therapy (**SDCT**).

Background

- On farms which carry out SDCT, treatment protocol based on **somatic cell count (SCC)** readings obtained from milk samples

- SCC readings used as a proxy for the concentration of leucocytes in milk - used to ascertain perceived risk of contracting mastitis

- It is advised that cows expressing **more than 200,000 cells/ml** in the milk should be administered antibiotic at dry-off.

Background

- Does blanket antibiotic treatment change the udder/milk microbiota, and consequently levels of antibiotic-resistant bacteria on-farm?

- Is there a relationship between treatment strategy at dry-off, the udder/milk microbiota and levels of antibiotic-resistant bacteria?
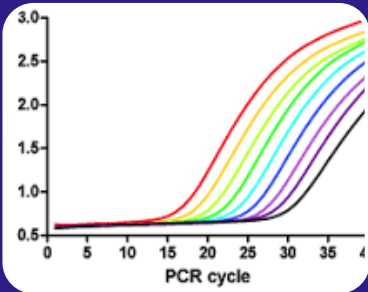
Experimental design
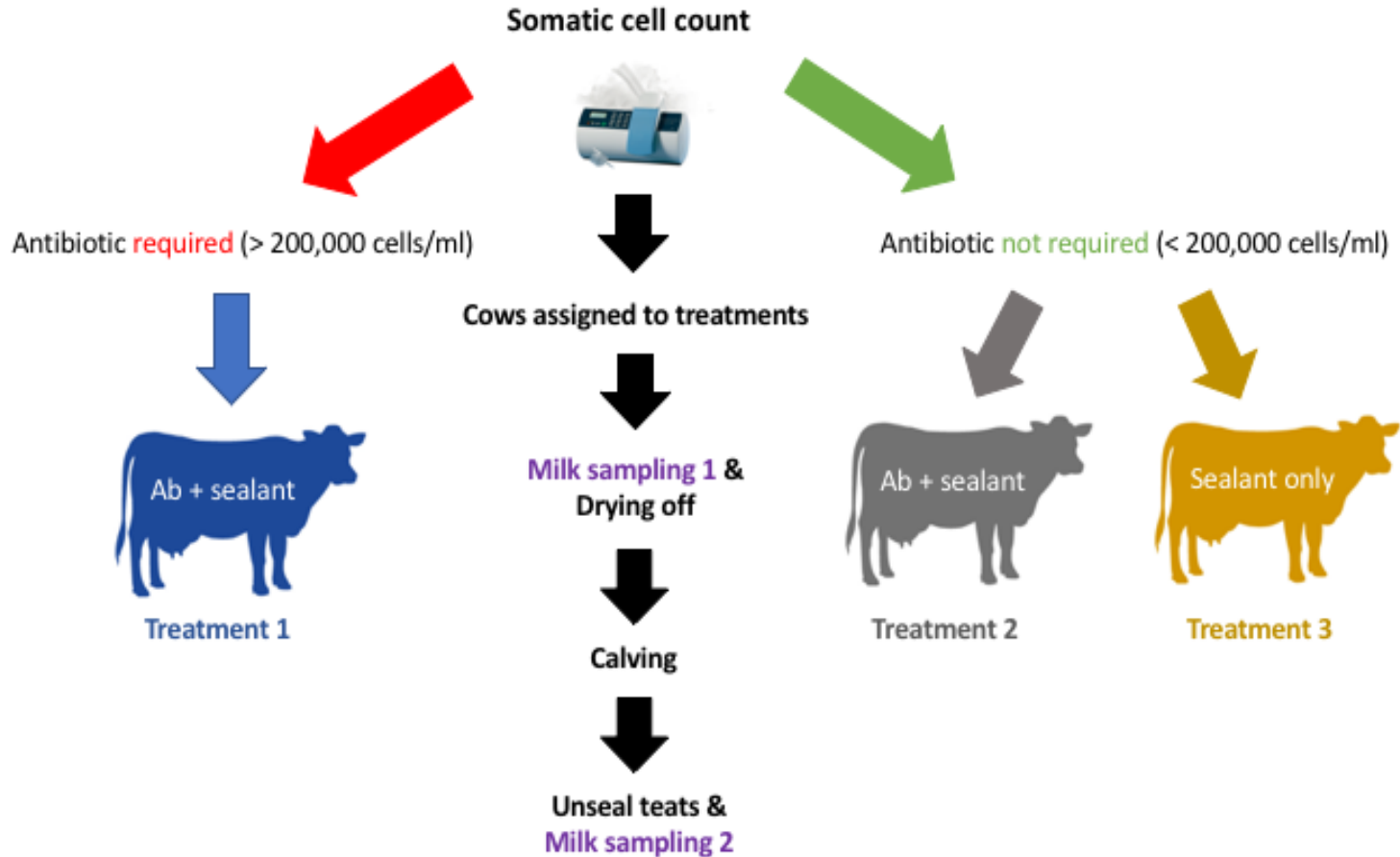
All milk samples were subject to (A) culturing, (B) DNA extraction and (C) storage at -80°C.
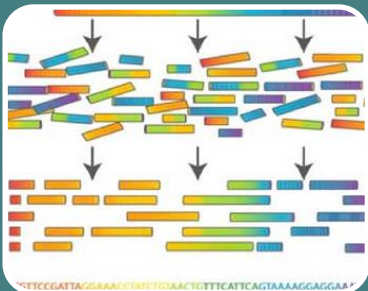
Measuring antimicrobial resistance

Antimicrobial sensitivity tests

- Culture milk sample and select single bacterial isolate
- Expose isolate to key antibiotics and measure zones of inhibition

Quantitative polymerase chain reaction (qPCR)

- Carry out DNA extraction using 1ml of milk
- Specifically amplify and quantify key genes associated with AMR

Whole genome sequencing

- Carry out DNA extraction using a single bacterial colony
- Shred up all DNA and sequence DNA fragments
- Use public databases to identify AMR genes

Antimicrobial sensitivity tests

- *E. coli* isolates (n = 40) were susceptible to all screened antibiotics, **except tetracycline**

- **22.5%** of the *E. coli* isolates screened were **resistant to tetracycline**

- **38.5%** of tetracycline-resistant *E.coli* were obtained from cows with a **high SCC**

- **Next steps:** Screen *E.coli*, streptococci and staphylococci isolates using antibiotics more specific to this unit – e.g. cloxacillin

AMR gene quantification

- The *tetQ* **gene**, linked to **tetracycline resistance**, was found in **32%** of the milk samples

- Antibiotic treatment associated with **4x increase** in *tetQ* gene compared to a **2x increase** in the sealant only group

- Interestingly, tetracycline has not been used on this unit for at least a decade…

- **Next steps:** Develop further qPCR assays targeting genes of direct relevance to this unit.

Whole genome sequencing

- The new data generated by antibiotic sensitivity testing will be used to inform selection for WGS

- Whole genome DNA sequences from *E.coli*, *Staphylococcus* and *Streptococcus* will be generated (n = 20, with a total of 60 isolates)

- This will allow us to fully explore AMR gene abundance and diversity in key mastitis-associated bacteria.

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