

# Evaluation of Impact of Antibiotic-Use Reduction Measures on the Prevalence of Antimicrobial Resistance and Pathogen Abundance in Pig Production Barns

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## Summary

The goal of this project is to compare the effect of the adoption of a RWA (raised without antibiotics) approach with non-RWA operations on the overall prevalence of antimicrobial resistance (AMR) and pathogen abundance in pig production facilities. We conducted surveillance of AMR and pathogens using whole genome sequencing (WGS), whereby primary data quantifying the resistome, virulome and bacterial diversity in the participating barns was obtained. Additionally, we developed a workflow methodology for metagenomics investigation of the effect of the RWA program on the resistome and virulome in conjunction with drug-use and animal health metadata collected from each type of barn.

## Introduction

In response to the general concerns about the spread of antimicrobial resistance (AMR) along with increasing public apprehension regarding the use of antibiotics in livestock production, various measures such as the total ban on use of antibiotics in livestock feed and strict regulations on any antibiotic use for treatment of sick animals were implemented in Canada. Another strategy available to producers include adoption of raised without antibiotic (RWA) production practices, wherein appropriate steps are implemented to completely eliminate antibiotic exposure of the pig from gestation to market, without compromising animal welfare. In this work, we seek to answer the question on how effective are these alternative strategies in reducing the total on-farm use of antibiotics, the occurrence of pathogens, and the prevalence of antimicrobial resistance?

To answer these questions, this study conducted longitudinal surveillance monitoring of farms that implemented a RWA program as well as conventional farms using antibiotics as prescribed by a veterinarian (non-RWA). The monitoring strategy focused on three key areas: antibiotics usage, antibiotic resistance, and prevalence of pathogens. Based on the findings, recommendations for best management practices will be developed to help ensure the success of intervention measures such as RWA or other similar alternative production programs.

## Experimental Procedures

For this study, we recruited two types of farms to participate: three (3) RWA farms and two (2) non-RWA farms. The overall workflow for the data collection and corresponding analysis to be conducted for this study is shown in Figure 1.

### *Activity 1 – Determining on-farm antibiotic usage patterns and total use*

Each participating farm was requested to share their inventory of antibiotics in their barn, and their record of the use of any antibiotics for treatment, including type of drug, dosage, type and number of animal(s) treated and approximate age, treatment cause, location in the barn, and date and time. Typically, producers collect these information as part of the CQA/CPE program, and so we requested for copies of these records every 3 months. Based on these collected data, the total antibiotic use and usage patterns were determined for each participating farm.

### *Activity 2 – Surveillance monitoring of prevalence of antimicrobial resistance and pathogens*

The second activity focused on monitoring the prevalence of antimicrobial resistance and pathogens in each of the participating farms. Representative fecal and manure samples were collected from each farm every 6 months from 6-week, 12-week and 20-week old pigs, and samples from the manure lagoon, and soil samples from the barn's immediate environment were also collected and analyzed. Sampling also included nasal swabs from 6-week old piglets, due to the potential for sequencing analyses to detect/identify subsets of respiratory viruses in addition to virulence factors along with other microorganism categories and their associated AMR.

## Results and Discussion

### *Activity 1 – Determining on-farm antibiotic usage patterns and total use*

Preliminary data obtained from the drug treatment records obtained from each participating barn from August 2018 to May 2020 indicate most antibiotics belong to four classes: Antifolates,  $\beta$ -lactams, Tetracyclines and Amphenicols. The most prevalent illnesses and treatment reasons recorded included: limping, scours, respiratory impairment and infection. Additional correlation analysis with resistome will determine if these illnesses/symptoms are related to antibiotic classes and whether it leads to any specific set or pattern of resistance genes.

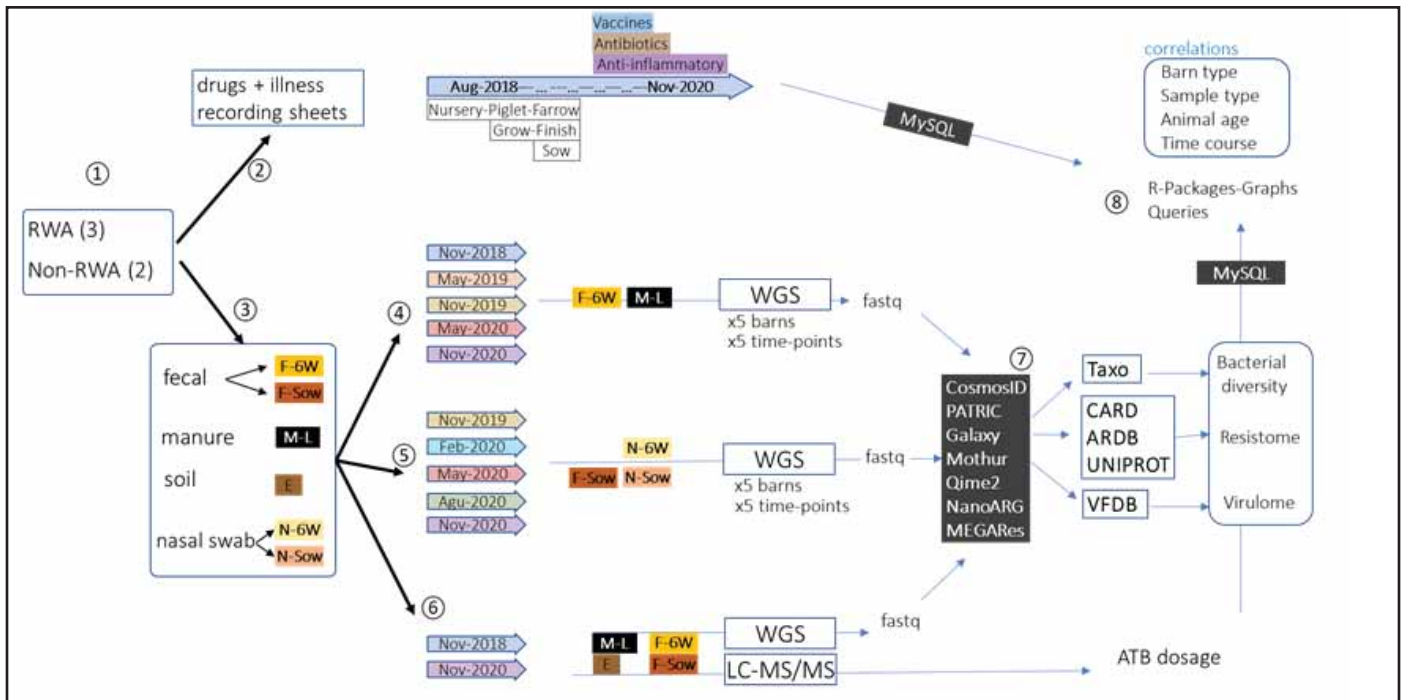
### *Activity 2 – Surveillance monitoring of prevalence of antimicrobial resistance (AMR) and pathogens*

Preliminary beta-diversity analysis of the resistome (statistical analysis between groups of samples) in 26 samples sequenced (three time-points from four barns and one time-point from an additional barn) showed two clusters of clearly-separate groups of type of samples – Fecal and Manure – with respect to the abundance of antimicrobial resistance genes (ARGs) (Figure 2). The Manure group had two close but distinct sub-groups that included the RWA and non-RWA data. Based on the first two time-points of this study, results demonstrate that comparative repeated measures of two ARGs readouts (abundance and frequency) significantly differentiate between RWA and non-RWA groups. For instance, we observed a significant decrease in the relative abundance of Tetracycline-ARGs and multi-drug resistant (MDR)-ARGs in manure samples from RWA barns. We also observed a significant decrease in the frequency of Tetracycline-ARGs in Fecal samples from RWA barns. On the other hand, a greater abundance of the Aminoglycoside-ARG class was observed in RWA barns. However, these observations remain to be confirmed in future sequencing time-points prior to correlation with drug usage trends.

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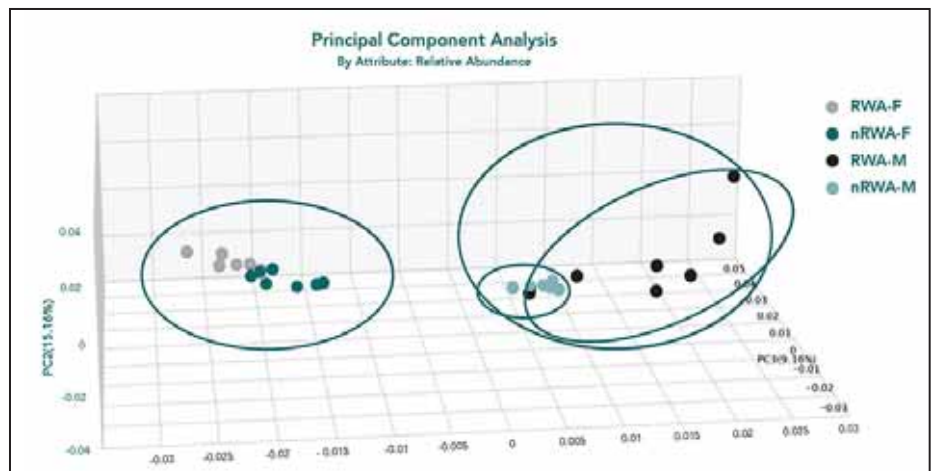
**Figure 1.** Diagram of workflow for longitudinal investigation of antibiotic resistance, pathogens and virulence factors associated with pig production. The diagram shows the steps in this project over a 2-year period of sampling and analyses: including sampling and whole-genome sequencing (WGS) strategy for Piglet (6-week-old) fecal material and manure every 6 months, sampling/WGS strategy for Piglet/Sow nasal swabs as well as Sow fecal samples every 3 months ((3,4,5), and first and last sampling time-points for WGS/LC-MS/MS strategy for Piglet/Sow fecal, manure and environmental samples (6). Raw shotgun data are analyzed comparatively through multiple platforms and with open source tools to generate 3 major classes of information: Bacterial Taxonomy, Resistome and Virulome (7).

**Implications**

Preliminary analyses demonstrated a substantial reduction in both MDR-ARGs and Tetracycline-ARGs in RWA barns as compared to non-RWA barns, suggesting that RWA measures can possibly contribute to mitigating the development of resistance to specific antibiotics used in pig production.

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**Figure 2.** Statistical analysis of the resistome - beta diversity showing 2 distinct groups: the left ellipse represents the fecal group (containing a non-distinct subgroup of RWA (RWA-F) and non-RWA (nRWA-F)). The right large ellipse represents the Manure group containing 2 close but distinct subgroups of RWA (RWA-M) and non-RWA (nRWA-M).