



# Antimicrobial resistance in bacteria of dairy cattle exposed to intramammary pirlimycin hydrochloride



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## BACKGROUND

- Mastitis is a very common and costly disease in dairy cattle
- Pirlimycin hydrochloride is an antimicrobial that is commonly used to treat mastitis in dairy cattle
- Use of intramammary pirlimycin alters the fecal microbiome in dairy cows
- There is little data on the unintended effects of intramammary antimicrobials on resistance profiles of microbes originating from the GI tract

## HYPOTHOSIS

Heifers treated with intramammary pirlimycin will have a higher proportion of antimicrobial resistant bacteria in their feces than those that were not treated.

## METHODS



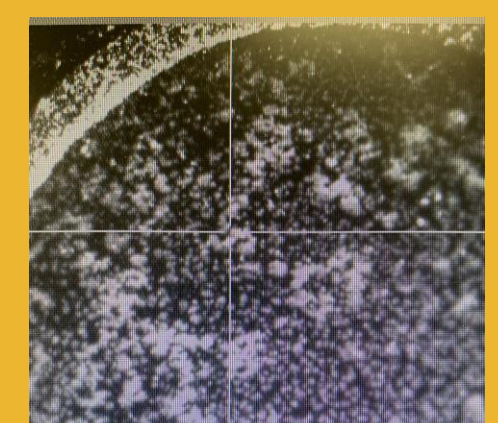
Feces were thawed at room temperature for 1 hour and then diluted at 1:100 with sterile saline.



100µL of the dilution was spread on to Mannitol Salt Agar, Columbia CNA agar, KF *Streptococcus* Agar and *Streptococcus* selection agar. 50 µL of the dilution was spread onto Columbia blood agar. All media were incubated at 37° C, 10% CO<sub>2</sub> and evaluated for growth at 24 and 48 hours.



*Staphylococcus* and *Streptococcus*-like organisms (*Streptococcus* species, *Enterococcus* species, *Lactococcus* species, *Aerococcus* species) were selected and isolated on Columbia blood agar.



MALDI-TOF was used to speciate each isolate.

## FECAL SAMPLE ORIGIN

- Fecal samples originated from University of Missouri Foremost Dairy heifers (n=24) that were enrolled in three groups: Control (n= 8) who did not receive pirlimycin treatment, Treatment group 1 (n= 10) received intramammary pirlimycin in one infected quarter once daily for two days at 24-hour intervals, and Treatment group 2 (n= 6) who received intramammary pirlimycin in one infected quarter once daily for eight days at 24-hour intervals.
- Fecal samples were collected once per day on day 0 (D0), D2, D7, D14, D21, and D28 and stored at -20° C until further analysis.

## PRELIMINARY RESULTS

- To date, 23 out of 120 fecal samples have been cultured for *Staphylococcus* and *Streptococcus*-like species. A total of 4 (n=21 isolates) *Enterococcus* species and 3 (n=7 isolates) *Staphylococcus* species have been identified to date.

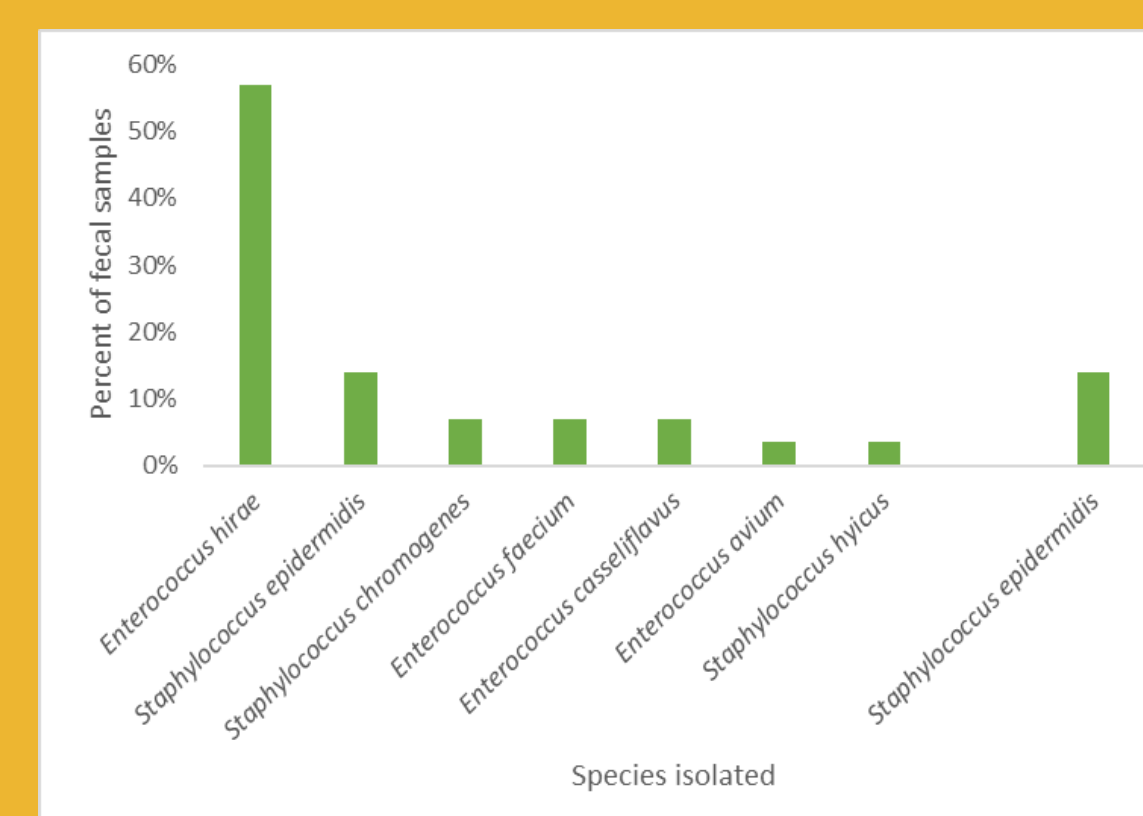


Figure 2. Percent of fecal samples (n =23) from both treatment and control groups containing various *Staphylococcus* and *Streptococcus*-like species isolated using selective media.

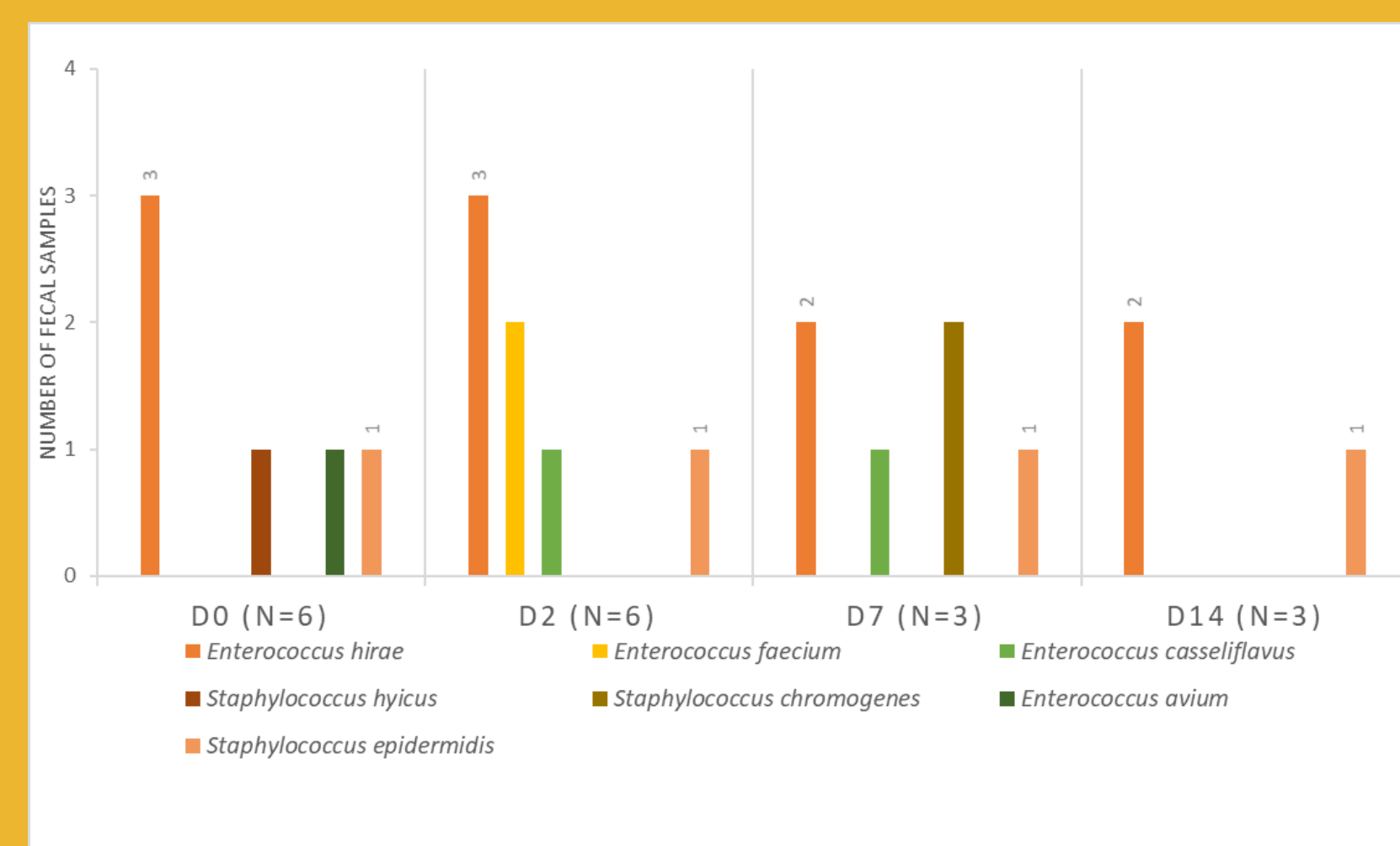


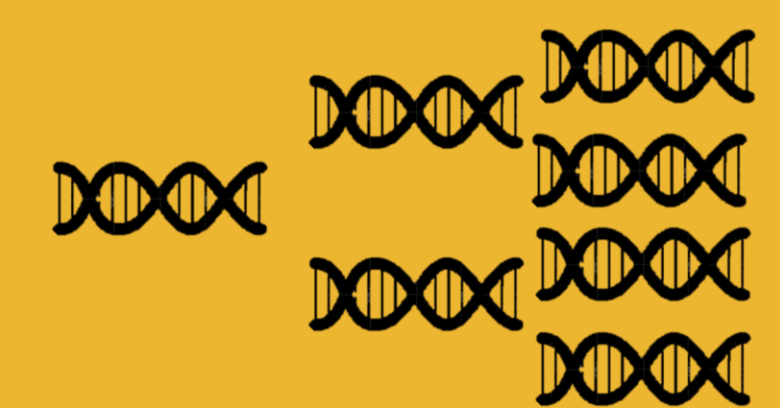
Figure 3. *Staphylococcus* and *Streptococcus*-like species isolated from 18 out of 50 fecal samples from treatment group 1 heifers before (D0) and after (D2, D7, and D14) treatment.

## DISCUSSION

- Next Phase:
  - ❖ Genotypic and phenotypic antimicrobial resistance of isolates will be determined using PCR and MIC
  - ❖ Data analysis will be conducted to determine if the level resistance differs between heifers in different treatment groups (treated or control) or over the course of treatment



MIC



PCR

- Impact of results:
  - ❖ The results of this study will help increase veterinarian's understanding of unintended risks involved in administering intramammary pirlimycin
  - ❖ This information can guide management practices regarding administration of pirlimycin and waste management protocols

## CONCLUSIONS

- The most common bacteria isolated from both the treatment and the control group was *Enterococcus hirae*
- 75% of isolates identified to date are *Enterococcus* species and only 25% are *Staphylococcus* species.
- Based on current data, it appears as though species richness is consistent on D0, D2, and D7 but decreased on D14

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