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Long-Read Metagenomic Analysis of Milking Filters as a Tool for Dairy Herds' Resistome Monitoring

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INTRODUCTION & AIM

The rapid spread of antimicrobial resistance (AMR) in humans, animals and environment poses growing challenges to global health. Rapid, cost-effective methods for routine resistome surveillance in dairy herds are increasingly needed to support antimicrobial stewardship and implement risk management strategies. High-resolution resistome profiling could be a possible solution to better understand antibiotic resistance landscape in dairy herds. This study aimed to provide a proof-of-concept for a quantitative analysis of the resistome retrieved from milking filters in six dairy herds in Lombardy (Italy).

METHOD

Milking filters were collected after routine milking. DNA was extracted using the PowerWater DNA kit (Qiagen, Hilden, Germany) and quantified with the NanoReady Touch series Micro Volume Spectrophotometer (Aurogene, Rome, Italy). DNA libraries were prepared using the SQK-RBK114.24 Rapid Barcoding Kit (Oxford Nanopore, Oxford, United Kingdom). Sequencing was performed on a MinION MK1C device; raw sequencing data were bascalled using Dorado v. 1.0.2 [1] and then analyzed with Abricate [2] and Centrifuge [3] bioinformatic softwares to uncover resistome and bacterial community composition of the different samples; NCBI AMRfinder plus [4] and the Johns Hopkins University CCB "bacteria and archea" were used respectively as databases for the analysis; finally results visualized using R statistical software.

RESULTS & DISCUSSION

The two most abundant families of resistant bacteria were Mycobacteriaceae and Staphylococcaceae for herd 1 and Moraxellaceae and Methylococcaceae for herd 5 while the other four herds didn't show predominante species harboring resistance genes or relevant levels of overall resistance. Two out of six dairy herds showed high levels of resistance, specifically herd 1 and herd 5 with 335 and 465 genomic copies/Gigabase (gc/Gb) when compared to the other four herds, which showed values lower than 20 gc/Gb. The most predominant levels of resistance were against gentamicin (17.6%) and kanamycin (15.1%) for herd 1 and spectinomycin (37.1%) and tetracyclines (18.8%) for herd 5.

CONCLUSION

This study shows a relatively quick and easy method to quantitatively analyze the resistome of milking filters based on a long-read sequencing approach. This approach can determine precisely levels of antibiotic resistance present in the sample and associate them with the bacterial families harboring them. Also, from this work emerges how milking filters could be used as an eligible matrix for antimicrobial resistance profiling of different dairy herds.

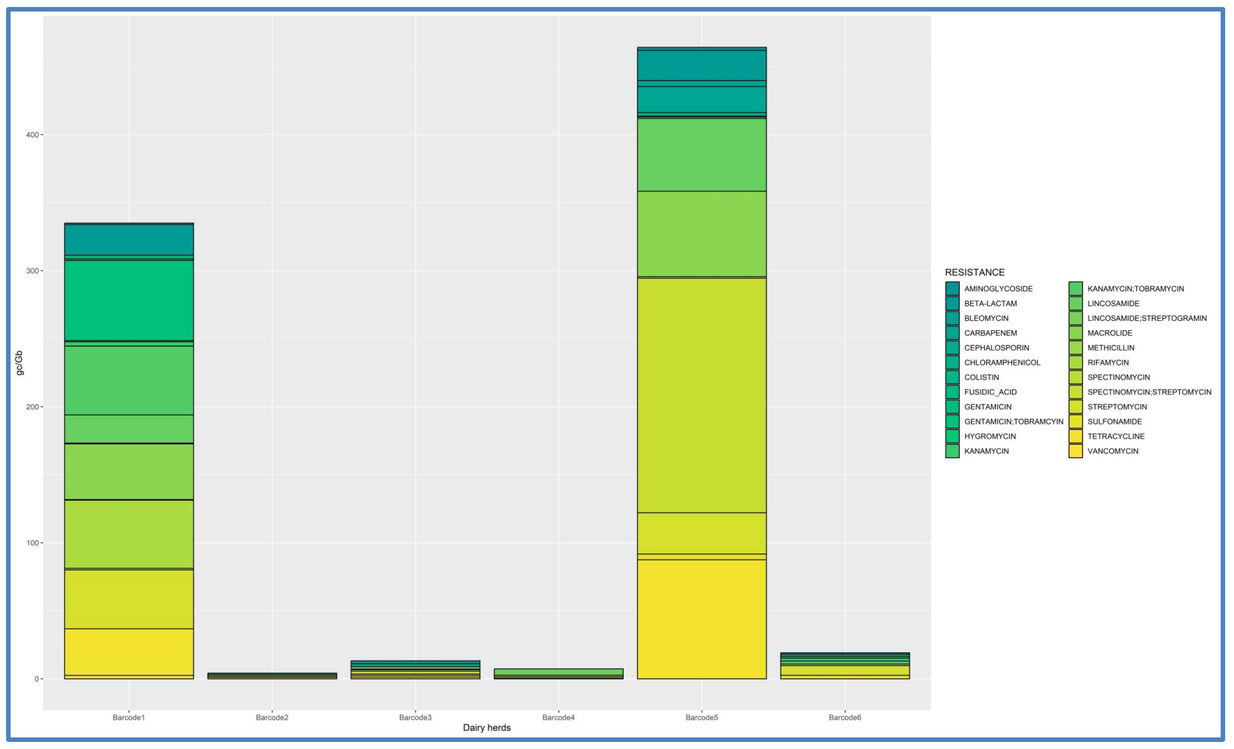

Figure 1: boxplot of antimicrobial resistance loads (gc/Gb) in milking filters samples divided by type of predicted phenotypic resistance. Viridis color palette used to enable visualization for colorblind people.

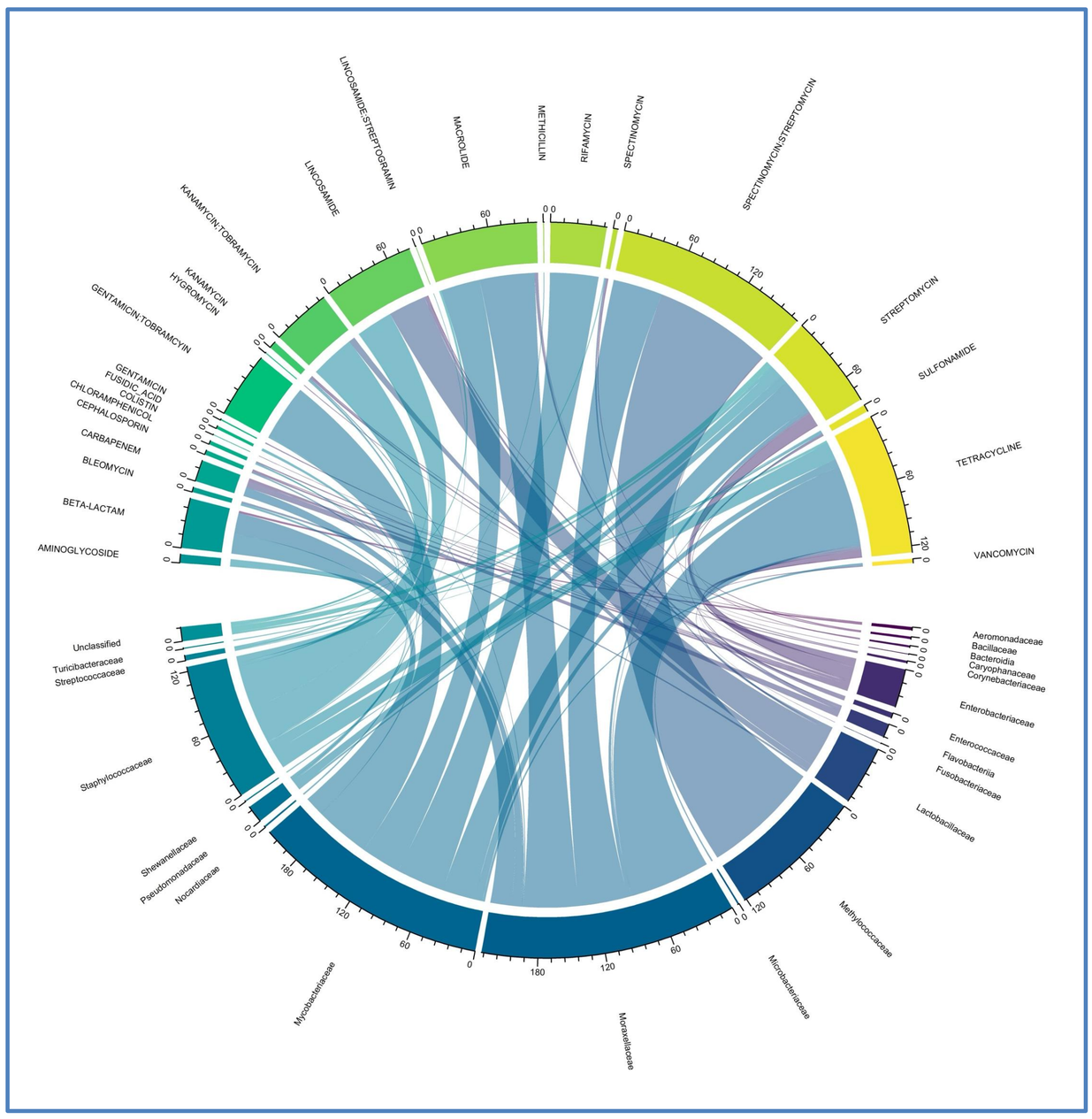

Figure 2: Chord diagram of overall antibiotic resistant bacterial families and relative mean antibiotic resistance genes load. Upper portion of the diagram shows predicted phenotypic antibiotic resistance and the lower portion shows antibiotic resistant bacterial families. Viridis color palette used to enable visualization for colorblind people.

FUTURE WORK / REFERENCES

1) Nanoporetech/Dorado 2025 2) Seemann T, Abricate, GitHub https://github.com/tseemann/abricate; 3) Kim D et al. Centrifuge: rapid and sensitive classification of metagenomic sequences. Genome Res. 2016 Dec;26(12):1721-1729. doi: 10.1101/gr.210641.116; 4) Feldgarden M et al. Validating the AMRFinder Tool and Resistance Gene Database by Using Antimicrobial Resistance Genotype-Phenotype Correlations in a Collection of Isolates. Antimicrob Agents Chemother. 2020 Mar 24;64(4):e00361-20. doi: 10.1128/AAC.00361-20;