OP48

Pilot study to predict the occurrence of foodborne pathogens in milk microbiome testing the animal sewage microbiome in a dairy cattle farm

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In 2022, the number of foodborne outbreaks in Europe increased by 43.9%, highlighting the urgent need to enhance surveillance systems and develop predictive tools for outbreak prevention. Monitoring foodborne pathogens in wastewater has emerged as promising and cost-effective approach to improve foodborne outbreak surveillance. Shotgun metagenomic analysis of human sewage has already proven successful in mapping the circulation of biological hazards and antimicrobial resistance genes within specific regions.

In this pilot study, we explored the feasibility of analyzing animal sewage from a dairy cattle farm to predict the milk microbiome and potentially prevent the entry of foodborne pathogens into the milk and cheese production chains.

A total of four sewage samples and four milk samples, of 50 ml each, were collected from the same farm during four longitudinal samplings conducted monthly between October 2023 and January 2024. Total DNA was extracted from all samples using a mechanical cell disruption followed by the PowerFood® Microbial DNA Isolation Kit. The extracted DNA was quantified using a BioSpectrometer® and subsequently fragmented and tagged with sequencing indexes and adapters using the Nextera XT DNA Library Preparation Kit. Shotgun metagenomic sequencing was then performed using the NextSeq 500 in paired-end mode (2x150 bp). The sequencing output ranged between 2.75 to 3.80 Gbp per samples. The alpha diversity index, showing the microbiome richness, varied across sewage samples, while it was homogenous for bulk milk samples. Beta diversity analysis clustered all the milk samples together while clustering of the sewage samples was in the same area of the PCoA plot. The predominant genera in sewage included Bacteroides, Prevotella, Alistipes and Pseudomonas. The prevalent genera prevalent in milk included Staphylococcus, Streptococcus Anaplasma and Enterococcus. Correlation analysis revealed a strong association (R>0.88) between specific bacteria species present in both sewage and milk samples, including Staphylococcus aureus which is a relevant foodborne pathogen in the dairy food system.

Keywords : microbiome, dairy cattle farm, sewage, foodborn pathogens, shotgun metagenomics