A NEXT GENERATION SEQUENCING APPROACH FOR ANTIBIOTIC RESISTOME SURVEILLANCE IN THE MILK PRODUCTION ENVIRONMENT.

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INTRODUCTION

Antimicrobial resistance (AMR) is globally recognised as one of the most serious threats to public health, with significant consequences for human and animal health and for the agriculture and food sectors. The widespread use of antimicrobial compounds in food-producing animals has attracted attention due to the potential transmission of resistant bacteria from food-producing animals to humans via direct contact or via the food chain. Antibiotics are used to treat bacterial infections in dairy cattle production systems; bovine mastitis, in particular, is recognised as the root cause of antimicrobial compound use in dairy farms worldwide. Among the different techniques applied to track AMR genes, Next Generation Sequencing technologies are promising tools. Given the current paucity of studies investigating the resistome of milk and its production environment through metagenomics technologies, the aim of this study was to describe the resistome of dairy farms with different historical somatic cells counts (SCC) using a shotgun metagenomics sequencing approach, whilst taking advantage of in-line milk filters.

METHODOLOGY

In May 2020, in-line milk filters were collected from the bulk tanks of five dairy farms with high historical SCCs and five dairy farms with low SCCs in North-West Italy; sampling was repeated in May 2021. A 10 g sample of each milk filter (N=20) was added to 90 ml sterile ringer's solution and homogenised. DNA was extracted and used for shotgun metagenomics to a sequencing depth of 50 M paired-end (PE) 2 × 150 bp reads on an Illumina NovaSeq 6000 platform. Sequencing data quality checks were carried out using FastQC, and filtering of raw data was performed using Trimmomatic; filtered reads were then mapped against the bovine reference genome with Bowtie2. High-quality, host-filtered reads were aligned to the MEGARes, CARD, ARG-ANNOT and Resfinder databases using BWA to perform a reads-based resistome characterisation; redundant genes were manually removed. Host-filtered reads were assembled de novo using IDBA-UD, and ABRicate was used on the generated scaffolds and metagenome assembled genomes (MAGs) to perform assembly-based resistome characterisation. Finally, WAAFLE was used to find novel horizontal gene transfer (HGT) markers in the assembled metagenomes.

RESULTS

Among the host-filtered reads, approximately 33,000 reads (0.5 % of all microbial reads) were associated with the AMR gene databases across all sample datasets. The assembly-based resistome characterisation revealed the presence of 10 AMR classes; aminoglycoside, antimicrobial peptides (AMPs), β-lactam, fosfomycin, fluoroquinolone, macrolide, lincosamide and streptogramin (MLS), multidrug (MDR) efflux pumps, phenicol, sulfonamide and tetracycline. Two more AMR classes were identified by the reads-based resistome characterisation, these were rifampin and triclosan. Out of 144 mobile genetic elements (MGEs) identified across 19 milk filters, 26 were identified as transposases, 1 was identified as a phage integrase and 4 were directly related to antimicrobial inactivation. The AMR classes were evenly distributed between the different sampling years and farms. The computational analyses allowed de-novo reassembly and reconstruction of 20 MAGs, which were further used to investigate the presence of AMR genes. Among the 18 MAGs identified, 8 carried AMR genes; in addition, mobile genetic elements (MGE) including plasmids were identified in two MAGs.

DISCUSSION

Understanding the distribution of AMR genes in a complex, important food matrix, such as milk, has relevance in terms of protecting consumers and maintaining high food safety standards. Milk filters have proven to be valuable tools that can be used to study the resistome in this production environment through the application of shotgun metagenomics sequencing. This approach facilitates the identification of numerous AMR determinants without the need for culture. In this context, de novo assembly allows for a more holistic AMR detection strategy, while the reads-based approach allows for the detection of AMR genes from low abundance bacteria that might be undetectable using assembly-based methods. It should be noted, however, that the reads-based approach may result in false positive prediction. The application of both reads-based and assembly-based approaches, despite being computationally demanding, has facilitated the comprehensive characterisation of a food chain resistome. At the same time, it has also allowed us to constructcomplete MAGs of importance to food safety andinvestigate MGE elements. Translating these findings into risk assessment outputs heralds the next generation of food safety controls.