NOVEL APPROACH TOWARDS FOOD SAFETY IN MILK: DETECTION OF ANTIMICROBIALS AND NON-TARGETED METABOLITE RESIDUES AS A PIVOTAL APPROACH TO PREVENTINGANTIBIOTIC RESISTANCE DIFFUSION.

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INTRODUCTION

For over 60 years, antibiotics have been widely used in animal husbandry for the prevention and treatment ofcommon pathologies. Moreover, the misuse of antibiotics to increase growth performance and feed efficiency can lead to the presence of antibiotic residues in milk. Concerns over antibiotic residues in food of animal origin arise due to the potential threat of direct toxicity to consumers, but mainly because low dosages of antibiotics could result in the alteration and possible development of resistant strains of bacteria. Regarding the above-mentioned main uses, the presence of residues in milk may be due to the miscellaneous use of antibiotics, either directly or indirectly (e.g., from farming and production environments), which represents a real threat to consumer health. This study aims to verify the absence of administered antimicrobials after therapeutic treatments, taking into consideration the withdrawal period. The targeted search for the previously administered antibiotics was implemented through a non-targeted search for their metabolites, which could still be pharmacologically active and interfere in the cheese-making process.

METHODOLOGY

141 raw bovine milk samples were collected from local farms located in the Piedmont Region of northern Italy, where most of the milk produced is used in Grana Padano PDO cheese production. The samples were all selected from dairy cows previously treated with different antimicrobial drugs due to medical conditions. The collection of milk was performed in accordance with the withdrawal period of all the drugs administered and, in this particular case, at the 7th milking. Confirmatory analyses were performed in duplicate according to the method described in our previous work. In brief, 1 mL of raw bovine milk, spiked at 2 ng mL−1 with the IS, extracted with 5 mL of McIlvaine buffer (pH 4.0) and 100 µL, 20% w/v of Trichloroacetic acid and then defatted with hexane, was purified by HLB SPE (Hydrophilic–Lipophilic Balance for Solid Phase Extraction). Analyses were performed by an HPLC system (Thermo Fisher Scientific, San Jose, CA, USA) coupled with a Thermo Q-Exactive Orbitrap (Thermo Fisher Scientific, San Jose, CA, USA). All the mass spectrometry

(MS) parameters for the full-scan acquisition (FS) were combined with the data-independent acquisition (DIA) for the MS2 response.

RESULTS

41 samples (29 %) showed the residual presence of a treatment, despite the fact that the withdrawal period was amply respected, with the sample collection taking place after the seventh milking. Moreover, in 9 % of the total samples, some compounds not indicated in the treatment protocol of the animal were detected, often also during screening tests. In particular, a major unexpected finding was that MRLs were exceeded in eight samples (20 % of the positive samples, or 6 % of the total samples). Antimicrobial metabolites were also found in all samples with the presence of enrofloxacin and lincomycin, three metabolites (ciprofloxacin, des-ciprofloxacin and des-enrofloxacin) for the detected quinolone and lincomycin sulfoxide for lincosamide. Enrofloxacin, according to the literature, is extensively metabolised into ciprofloxacin and other minor metabolites, with the former still retaining antimicrobial activity. Two new metabolites that had not been reported in the literature were also tentatively identified and presented for their chemical structure.

DISCUSSION

Regarding the new metabolites, the ion with m/z 334.1198 including its MS2 spectrum can be attributed to a compound resulting from the break down of the piperazine ring. This compound, entitled 1-cyclopropyl-6-fluoro-7-((2-(methylamino)-2-oxoethyl amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) according to IUPAC nomenclature, is abbreviated as ENRO-N-methylacetamide. The second metabolite (5-(1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-2-hydroxy-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxamido)-2-oxopentanoic acid), named ENRO-ornithine (m/z = 491.2311), proved to be essential as it was similar to enrofloxacin itself. Some metabolites of Enrofloxacin are cleared from plasma to milk, for example lysine, but ornithine conjugate has not been reported so far. In particular, ciprofloxacin, des-ciprofloxacin, des-enrofloxacin, ENRO-N-methylacetamide and ENRO-ornithine were found in one sample treated with enrofloxacin, in which the parent drug was found at a higher concentration (25.50 ng mL−1). The detection of ciprofloxacin and ENRO-ornithine in one sample not declared for enrofloxacin treatment, in which the parent drug was detected at 0.20 ng mL−1 represents an important finding.