



POSTER

ASSESSMENT OF THE HYGIENIC AND SANITARY STATUS OF RAW-MILK MOUNTAIN CHEESES THROUGHOUT THE CHEESEMAKING PROCESS AND EVALUATION OF ANTIMICROBIAL RESISTANCE PHENOMENA

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Purpose. Traditional production of raw milk cheese in small scale integrated livestock mountain huts is a widely spread practice in Alpine regions. However, it is possible for resulting products to harbor a variety of harmful microorganisms. Microbiological safety of raw milk cheeses is a highly controversial topic: several studies report several food-borne outbreaks that are related to consumption of cheeses produced with unpasteurized milk. Moreover, the presence of antibiotic-resistant pathogenic bacteria in food may lead to hard-to-treat foodborne infections. This study investigates the sanitary quality and antimicrobial resistance (AMR) profile among the production of mountain huts raw milk cheeses.

Methods. A total of 6 raw milk, 6 curd and 5 raw milk uncooked cheese, with at least 50 days of ripening, samples were collected from 6 producing cow's milk cheese mountain huts in the Alpine region of Italy and subjected to microbiological analyses. 4 *Escherichia coli*, 4 Shiga toxin-producing *Escherichia coli* (STEC) and 8 coagulase-positive staphylococci strains, isolated from different matrices, were tested for AMR using the broth microdilution method with automated determination of the minimum inhibitory concentration (MIC). For both veterinary and human panels, tested classes of antibiotics were Beta lactam, Fluorquinolone, Macrolide, Aminoglycoside, Lincosamide, Rifamycin, Sulfonamide. Additionally, human panels also included: Tetracycline, Polymixin, Fusidane and Oxalidinone. Breakpoints were established according to EUCST, CLSI and CASFM guidelines.

Results. *Salmonella* spp. and *L. monocytogenes* were not detected in any of the samples. Milk showed low counts of

E. coli (<1 CFU/ml) and variable levels of coagulase-positive staphylococci from <10 CFU/ml up to 8,7x10² CFU/ml. By contrast, the curd showed greater concern: staphylococci increase with an average of approximately 40-fold compared with milk and *E. coli* showed mean values of 2,1x10² CFU/g in mountain hut n. 3 and 1,6x10³ CFU/g in mountain hut n. 4, combined respectively with the detection of STEC in the curd. STEC presence in cheese of mountain hut n. 4 was also detected after 50 days of ripening. Overall, antimicrobial resistance data from 4 *E. coli*, 4 STEC and 8 coagulase-positive staphylococci strains showed a predominant susceptibility to antibiotics tested using the veterinary panel. 9% of the resistance patterns detected were mainly associated with beta-lactams, particularly ampicillin (60%), to which all *E. coli* strains isolated from curd samples from 4 different huts, as well as the STEC strains isolated from the only cheese in which they were detected, were resistant. Coagulase-positive staphylococci strains, isolated from milk and curd samples from 4 different huts, showed resistance (50%) to ampicillin and penicillin. AMR data obtained using human panels of antibiotics showed almost complete susceptibility to the antibiotics tested. The same human panels also showed resistance to benzylpenicillin in 4 coagulase-positive staphylococci strains isolated from 3 different huts. Conclusions: Overall, the findings are promising regarding the main pathogens investigated even if attention is required regarding processing hygiene, curd control, and the management of mountain huts with STEC-positive results. From an AMR perspective, the overall profile indicates good efficacy of the antimicrobials tested, while highlighting specific concerns regarding beta-lactams.