

AMR patterns and RAPD profiles of *Streptococcus uberis* strains isolated from a clinical bovine mastitis outbreak

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Introduction

Streptococcus uberis is globally recognized as an environmental mastitis pathogen but some strains show a contagious transmission (Wald et al., 2020). Molecular genotyping contributes to understand their modes of dissemination and represents an important tool in epidemiological studies of *Strep. uberis* mastitis. Random Amplified Polymorphic DNA (RAPD) PCR is a rapid and inexpensive subtyping technique to detect differences in *Strep. uberis* (Zadoks et al., 2003). This method was shown to be efficient in identifying clonal strains carried by cows within a farm, whose high prevalence could suggest their role in the intramammary infection spreading (Tomazi et al., 2019). The antimicrobial therapy is still the main strategy for their control and treatment, but the widespread use of drugs has led to an increasing diffusion of *Strep. uberis* antimicrobial resistance (AMR), resulting in several cow health problems (Pol and Ruegg, 2007).

The antimicrobial susceptibility and the genotypic variability of *Strep. uberis* strains isolated from a bovine clinical mastitis (CM) outbreak were determined with the aim to investigate the dynamics of *Strep. uberis* infections at herd level.

Materials and methods

A retrospective cohort study was undertaken in November of 2019 within a single dairy farm, located in Northern Italy. During the 1-month study period, the herd exhibited a high prevalence of *Strep. uberis* CM, whose percentage of new clinical cases was 28%. A total of 26 *Strep. uberis* isolates were obtained from the quarter milk samples of 26 cows with CM. The identification of *Strep. uberis* strains at the species level was confirmed by using the MALDI-TOF MS. As only isolates with MALDI scores >2.0 were eligible to be selected in this study, 3 isolates were classified as other streptococci and excluded. The remaining 23 isolates identified as *Strep. uberis* were typed by RAPD-PCR, performed with primers ERIC1 (5'-ATGTA-AGCTCCTGGGGATTAC-3'), D11344 (5'-AGTGAATTCGCGGTCA-GATGCCA-3') and D8635 (5'-GAGCGGCCAAAGGGAGCAGAC-3'). All were tested against a total of 14 drugs (amoxicillin/clavulanic acid, ampicillin, cefazolin, ceftiofur, cefoperazone, cequinome, enrofloxacin, florfenicol, erythromycin, lincomycin, oxacillin, penicillin, tetracycline and trimethoprim/sulfamethoxazole), by using minimum inhibitory concentration (MIC) assay.

Results

The *Strep. uberis* strains had different degrees of resistance to the 14 active substances, but all were resistant to lincomycin and most (87%) also to tetracycline. The percentage of the isolates with a multidrug resistance (MDR) phenotype was 21.7%, with 8.7% of the isolates having intermediate resistance to penicillin and the same percentage to florfenicol; one isolate showed complete resistance for enrofloxacin and another one for oxacillin. We carried out cluster analysis to understand strain heterogeneity. As strains with a similarity coefficient equal to or higher than 90% can be considered as closely related, the combination of RAPD-PCR profiles obtained with the 3 primers displayed a conserved pattern within the herd. At 90% similarity, 3 distinct clusters were detected and most (52%) of the strains were grouped in the same cluster. The remaining 48% of *Strep. uberis* strains were divided in 2 minor genotypic clusters or did not enter any cluster.

Significance

Phenotypic results revealed that AMR was widespread and MDR strains were present but not prevalent. Surveillance data can help with the identification of the most appropriate antibiotic agents for taking adequate control and treatment protocols. Genotypic analysis suggested that contagious transmission of *Strep. uberis* mastitis in the investigated CM outbreak could be linked to a restricted number of cow-adapted strains. The relevance of this work consisted in the identification of strains likely capable of cow-to-cow spread, that may be meaningful for practical management.

