ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI SCIENZE MEDICHE VETERINARIE

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CURRICULUM: Produzioni animali e sicurezza alimentare
Anno di attività: [3°]

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Part 1: Effect of precision diagnostics for mastitis control

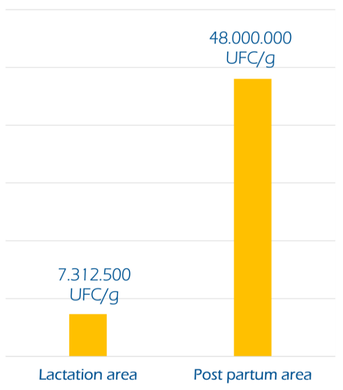
Aims: Improving mastitis control into the University of Bologna dairy farm, through the application of a protocol for precision diagnostic. Epidemiological survey on *S. uberis* and better usage of antimicrobials for mastitis treatment.

Materials and Methods: 1) Streptococcus uberis isolation from environmental samples coming from different areas (n=120) and enumeration: bedding, drinking water, feed, animal skin, faeces, milking machine and floor of milking parlor; 2) Bacterial isolation from milk samples obtained at mammary quarter level of 113 lactating cows across lactation: i) when showing clinical or subclinical mastitis symptoms, ii) after mastitis treatment; 3) Bacterial isolation from milk samples obtained at mammary quarter level of 113 lactating cows: i) at post-partum (10 days after parturition) and ii) at dry-off; 4) Antimicrobial susceptibility evaluation (Kirby-Bauer).

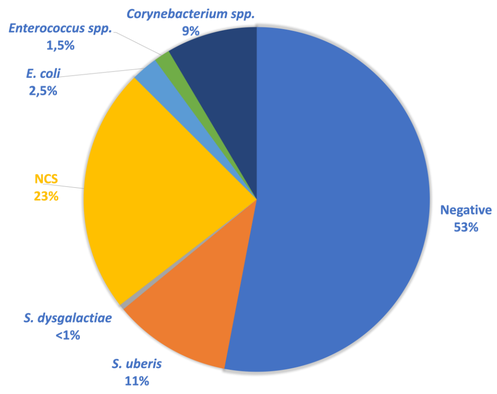
Results: S. uberis was isolated from bedding, drinking water, animal skin, milking machine and floor of milking parlor, while faeces from rectal ampulla repeatedly resulted negative; Post partum area was the most critical for environmental enumeration of S. uberis ($p \leq 0,05$); Most frequently isolated microorganisms from milk samples were NCS (23%) and S. uberis (11%); Milk samples at post-partum had an higher prevalence (11%) of S. uberis than at dry-off (5%); High recovery rate (85%) in animals treated with amoxicillin/clavulanic acid and 1st gen. cephalosporine; S. uberis resulted susceptible to the majority of antimicrobials

Conclusions: The results of this work confirm, as reported in literature, that post-partum is the most critical area for mastitis held by S. uberis, furthermore microbiological monitoring of cows in early lactation and a selective treatment improved the control of S. uberis mastitis, this approach should be associated with an improved environmental management; though a longer observation period is needed to properly evaluate the efficiency of this protocol.

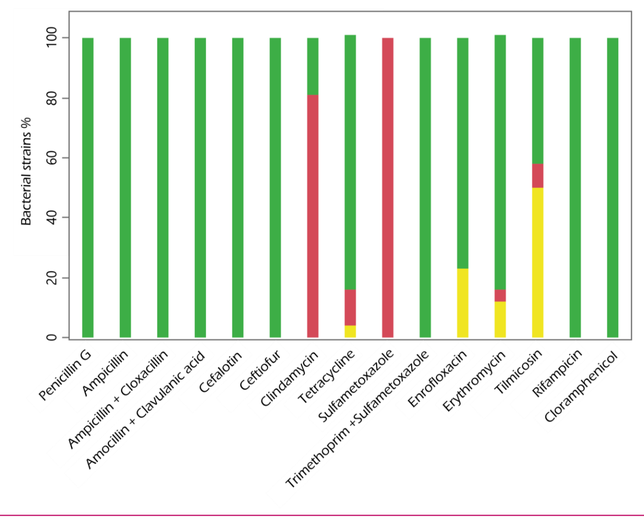
S. uberis enumeration in different areas



Results of microbiological analysis of milk samples



S. uberis antimicrobial susceptibility



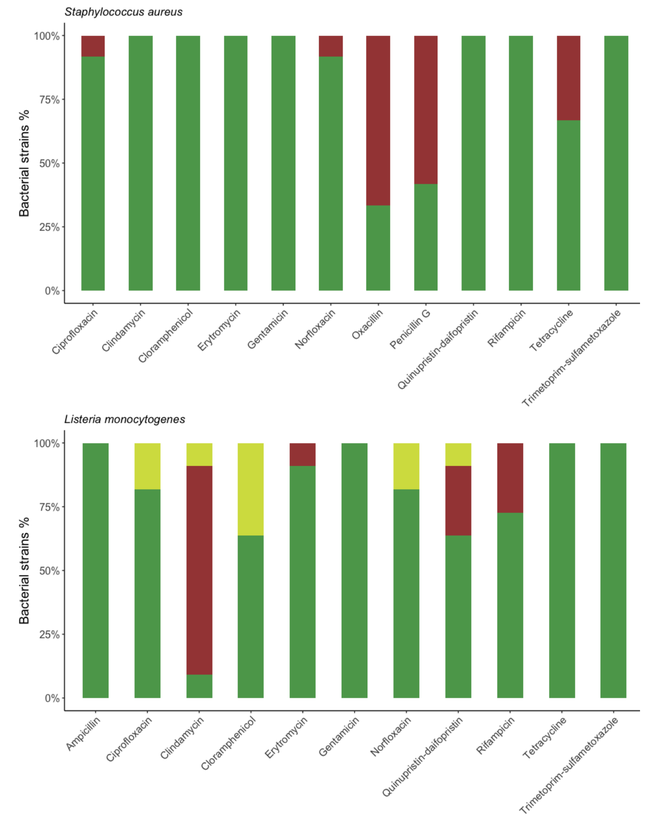
Part 2: Pathogens in artisanal food products, in situ and in silico approach

Aims: Characterization of pathogens isolated from artisanal cheeses and fermented sausages. Development of an exposure assessment to evaluate the exposure of consumer to *Listeria monocytogenes* during the shelf life of this products, whit focus on fresh goat cheeses, due to the recent listeriosis outbreaks reported in Spain for this product.

Materials and Methods: Microbiological analysis: 1) Pathogens isolation (ISO methods) from environmental (n=144), raw ingredients (n=60) and products (n=60) samples, coming from different (n=4) enterprises producing cheeses and fermented sausages; 2) Strains identification and characterization by MALDI-TOF and 16s rRNA; 3) Antimicrobial susceptibility evaluation (Kirby-Bauer)

Exposure assessment: 1) Data mining from literature and industry databases for growth models and temperature-time profiles; 2) Modular Process Risk Model methodology, designed using temperature-time profiles of a real distribution chain and built using the *R software;*

Antimicrobial susceptibility patterns



Results: Microbiological analysis: *L. monocytogenes* was detected in 10, 16.6 and 13.3% of raw meat, Salchichón and environmental, respectively. PCR results indicated that all raw meat isolates belong to serotype 4b, 4d and 4e and all environmental isolates belong to serotype 1/2a. Regarding Salchichón isolates, 1/2a, 1/2b, 4b, 4d and 4e serotypes were identified, indicating that the products could have been contaminated by both raw meat and environment. *Staphylococcus aureus* was detected in 6.6% of both milk and cheese samples, an in 16.6 and 10% or raw meat and Salchichón. Both pathogens were sensible to most of the tested antibiotics, excepting clindamycin, to which the majority of *L. monocytogenes* were resistant, and oxacillin and penicillin to which the majority of *S aureus* were resistant.

Exposure assessment: 1a) Data for temperature-time profiles were obtained from literature and from an artisanal enterprise producing fresh goat cheeses; 1b) A model for the growth of *L. monocytogenes* in fresh goat cheeses, developed in the context of this project, was used to predict its fate during transport and storage; 2) The implementation of the growth model and temperature-time profiles in the modular exposure assessment, highlighted how under rutinary-conditions of the distribution chain, the maximum concentration of *L. monocytogenes* can be reached already in the first phases, due to its growth ability in fresh goat cheeses.

Conclusions

The results of this study deepen knowledge on serotype variability and antimicrobial resistance *of L. monocytogenes* and *S. aureus* strains found in artisanal cheeses and fermented sausages enterprises. The results of the exposure assessment indicated that food distribution chain conditions, for fresh goat cheeses, enable *L. monocytogenes* growth along transport and retailing, in line with the regularity of the outbreaks. This led to consider that focused control measurements should be implemented to reduce risk of growth of this pathogen in this type of products.