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## Effect of inactivated cultures of *Lactobacillus rhamnosus* and antibiotics on subclinical mastitis quarter milk microbiota.

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Water buffaloes mastitis represents a major issue in terms of animal health, cost of therapy, premature culling and decreased milk yield. The emergence of antibiotic resistance has led to investigate strategies in order to avoid or minimize the antibiotic use, especially during subclinical mastitis (SM) (Moroni et al., 2006). *Lactobacillus rhamnosus* is part of the normal gut microflora, having meanwhile an immunostimulatory activity (Fong et al., 2016). The aim of this study was to investigate the change of milk microbiota after the therapeutic treatment of mammary gland quarters affected by subclinical mastitis with inactivated cultures of *Lactobacillus rhamnosus* and antibiotics. A number of 43 quarters from 20 pluriparous animals and affected by subclinical mastitis (SM), with no signs of clinical mastitis and aerobic culture positive for udder pathogens were included in the study; milk samples were positive for coagulase-negative or positive *Staphylococci* and/or *Streptococcus agalactiae*. A total of 11 quarters were locally treated with antibiotic (amoxicillin trihydrate), 15 with *Lactobacillus rhamnosus* and 17 with PBS as negative control, by means of intramammary injection. Samples were collected at two time points, T<sub>0</sub> (pre-treatment) and T<sub>5</sub> (after 5 days post-treatment) and V<sub>4</sub> region of 16S rRNA gene was amplified by PCR and sequenced using Ion Torrent Personal Genome Machine. The software Quantitative Insights Into Microbial Ecology (QIIME version 2) was used to analyse data. Microbiota composition was evaluated in terms of taxonomy at phylum, family and genus level. Microbiota structure was investigated through alpha and beta diversity analysis which take into account differences within and among samples, respectively. Non-parametric test, namely Wilcoxon signed rank and Kruskal-wallis followed by Dunn test, were used to perform statistical analysis for paired and unpaired groups, respectively.

Regarding taxonomy, the microbiota composition of SM quarters showed no major changes after PBS treatment, while differed after antibiotic treatment where *Staphylococcus* decreased its relative abundance from 41% at T0 to 3% at T5. *Lactobacillus rhamnosus* induced a less dramatic change in milk microbiota, although the relative abundance of some genera was found to be modified, among which an increase of *Pseudomonas* from 1.5% at T0 up to 4% at T5. The taxonomy at genus level is shown in Figure 1. However, beta diversity showed no differences between the microbiota structure of quarters treated with *Lactobacillus rhamnosus* and PBS (T0 vs T5). The effect of the treatment was different between antibiotic- vs PBS- treated groups and antibiotic- vs *Lactobacillus rhamnosus*- treated groups. In conclusion, this study allowed to characterize the microbiota in milk from animals treated with *Lactobacillus rhamnosus* and antibiotics; while changes in milk microbiota after antibiotic treatment were evident, changes after *Lactobacillus rhamnosus* were more limited. Following investigation will include the study of the microbiota changes of antibiotic-treated quarters during more than two time points, in order to investigate the colonization of mammary gland after antibiotic treatment during a time course.

**Figure 1:** Microbiota composition at genus level (relative abundance > 1%).

SM\_PBS\_T0: subclinical quarters treated with PBS (pre-treatment)

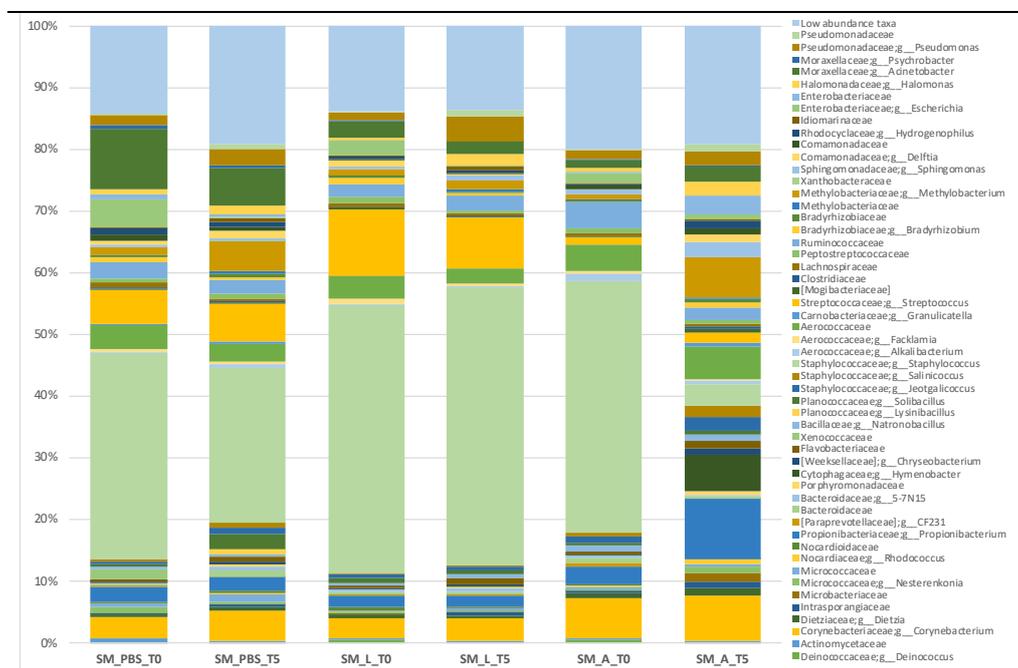
SM\_PBS\_T5: subclinical quarters treated with PBS (post-treatment)

SM\_L\_T0: subclinical quarters treated with *Lactobacillus rhamnosus* (pre-treatment)

SM\_L\_T5: subclinical quarters treated with *Lactobacillus rhamnosus* (post-treatment)

SM\_A\_T0: subclinical quarters treated with antibiotic (pre-treatment)

SM\_A\_T5: subclinical quarters treated with antibiotic (post-treatment)



## References

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