Staphylococcus aureus and *Streptococcus* Mastitis
Summary

Introduction
This summary provides an overview of the updated Disease and Product analysis for *Staphylococcus aureus* and *Streptococcus* infections of the mammary gland, prepared by DISCONTOOLS experts. The report reviews current knowledge, disease control tools, existing gaps, and necessary research for improved diagnostics, vaccines, and treatments. See www.discontools.eu for full details and reports.

Disease Profile
The gram-positive bacteria *Staphylococcus aureus* and streptococci (mainly *Streptococcus agalactiae*, also known as Group B *Streptococcus* or GBS, *Streptococcus uberis,* and *Streptococcus dysgalactiae* subsp. *dysgalactiae*) are leading pathogens responsible for mastitis in dairy animals, including cows, sheep, goats, camels, and buffaloes. Mastitis, an inflammation of the mammary gland, is the costliest disease in dairy production, leading to significant economic losses. It can manifest in subclinical or clinical forms, both of which significantly reduce milk yield. *Staphylococcus aureus* can persist intracellularly, form biofilms, spread contagiously, and establish chronic infections, which are associated with poor antibiotic treatment success rates, although excellent control of this organism has been achieved in many intensively managed dairy systems. Streptococci can spread through contagious and environmental transmission routes, depending on species and management system. In contagious transmission, the primary reservoir is the infected mammary gland, whilst milking enables bacterial spread through contaminated equipment, milkers' hands, and infected milk. Environmental reservoirs, including manure, bedding, and water also contribute to exposure. Mastitis remains endemic in dairy herds worldwide, with prevalence influenced by region, herd management, and biosecurity practices. Some bacterial strains display host adaptation, with dominant clones circulating within herds, further complicating control efforts.

Zoonotic risks and outcomes
Mortality due to *S. aureus* mastitis is rare in dairy cows but can be significant in heifers, ewes, and goats, especially in cases of peracute (gangrenous) mastitis. Chronic infections frequently lead to premature culling, contributing to economic losses and reduced herd productivity and sustainability. Early detection and control measures are essential to improving treatment outcomes and minimizing antimicrobial use (AMU) and the long-term impact on herd udder health. Although human infections from bovine-adapted strains are uncommon, concerns persist about interspecies transmission and antimicrobial resistance (AMR), particularly with methicillin-resistant *S. aureus* (MRSA), which poses potential public health risks. The dynamics of the transfer of *S. aureus* or *S. agalactiae* strains between humans and cattle deserve to be investigated regularly using modern genotyping methods, such as whole genome sequencing.

Diagnostics
Many diagnostic tools are available for detecting *S. aureus* and *Streptococcus* mastitis, including laboratory-based or on-farm bacterial culture, and molecular techniques. Bacterial culture is still seen as the gold standard for the diagnosis of intramammary infections, although it requires incubation time and careful sampling to prevent contamination. On-farm culture methods are available for rapid diagnosis. Molecular methods such as PCR and LAMP enable faster pathogen detection but cannot distinguish live from dead bacteria, potentially leading to false positives. MALDI-ToF MS is increasingly used for species identification of cultured isolates in diagnostic labs, leading to identification of hitherto unrecognized species for which little information on transmission or treatment is available. Milk somatic cell count (SCC) is widely used as indicator of inflammation but lacks pathogen specificity, as do other markers of inflammation. On-farm tests can differentiate some groups of bacteria, but require careful validation and

interpretation. Remaining challenges include the need for rapid, cost-effective, and accurate cow-side tests that can differentiate between infection and contamination, and standardized international guidelines for diagnostic consistency. Improving diagnostic tools will enhance early detection, guide targeted treatments, and contribute to better mastitis control while reducing reliance on antimicrobial use.

Vaccines

Commercial mastitis vaccines are available in several countries, including inactivated (*S. aureus*) and subunit (*S. uberis*) vaccines. These vaccines primarily stimulate humoral immunity and have shown variable efficacy, with some independently peer-reviewed scientific studies reporting reduced disease severity, while others found no significant benefit. None of these vaccines provide sterile immunity, and their effectiveness in preventing new infections is limited. There is a lack of independent large-scale field trials assessing vaccine efficacy under different management conditions. There is an urgent need to better understand host-pathogen interactions and the host's capacity to develop true protective immunity, and to identify reasons behind the failure of previous vaccine attempts. Future vaccine development should prioritize enhancing cell-mediated immunity and identifying conserved protective antigens. Innovative approaches, such as subunit vaccines, viral vector-based immunization, and RNA or DNA vaccines, should be actively explored, as should the impact of adjuvants and administration routes on the effectiveness of mastitis vaccines. Finally, clearly defined outcome criteria are essential for testing and validating new vaccines, alongside independent scientific evaluations of their efficacy in field trials.

Pharmaceuticals and Treatment

Treatment success is highly variable but risk factors for cure are well-known and largely consistent across pathogen species. Increased age of the animal or the infection and AMR of the pathogen indicate poorer outcomes. Cure after treatment may be more likely in the dry period than in lactation, but dry cow therapy may fail in chronic cases. New strategies focus primarily on pathogen-targeted therapy or alternatives to antimicrobials, e.g., bacteriophages and endolysins, which may also work in biofilms. Key challenges include high development costs, regulatory restrictions, the narrow host range of phages, and limited commercial incentives. With growing concerns over AMR, research into biologicals, immunotherapies, and advanced diagnostics is essential for optimizing mastitis management while minimizing AMU.

Knowledge Gaps

Significant research is still needed to better understand host-pathogen interactions and the underlying mechanisms of protective immunity, which are crucial for the development of effective vaccines. Other potential elements of long-term control strategies include further improvement of genetic resistance to mastitis in dairy cattle and immunomodulatory interventions, such as host-directed therapies that modify essential cellular processes involved in pathogen survival or replication, as well as those that target the host immune response to enhance immunity or mitigate immunopathology. Furthermore, there is a need for rapid, sensitive, specific, affordable, and user-friendly cow-side diagnostic tests for early detection of mastitis and its causative agents. This would help to guide cow- and herd-level interventions based on the risk of transmission, the likelihood of cure, and the benefit of treatment. In high-income countries with well-developed veterinary services and diagnostic laboratories, current knowledge on control of contagious transmission has largely been sufficient to limit the prevalence of *S. aureus* and *S. agalactiae* mastitis, and there is an ongoing need for uptake of existing knowledge and control measures.

Conclusion

Mastitis caused by *S. aureus* and *Streptococcus* remains a significant challenge in dairy production. Effective control requires advancements in uptake of current knowledge, new diagnostic tools, the development of novel effective vaccines, and alternatives to antimicrobials.