

Molecular characterization, antimicrobial resistance and virulence genotyping of *Staphylococcus aureus* from subclinical mastitis dairy cows

Ntelekwane G Khasapane¹, Sebolelo J Nkhebenyane¹, Zamantungwa TH Mnisi,^{2 & 3} Oriel Thekiso⁴

¹ Centre for Applied Food Safety and Biotechnology, Department of Life Sciences, Central University of Technology, 1 Park Road, Bloemfontein, 9300, South Africa;

² Vectors and Vector-borne Diseases Research Programme, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, Pretoria, South Africa;

³ DSI-NRF SARChI in Ecosystem Health, Department of Biodiversity, University of Limpopo, Sovenga, 2797, South Africa.

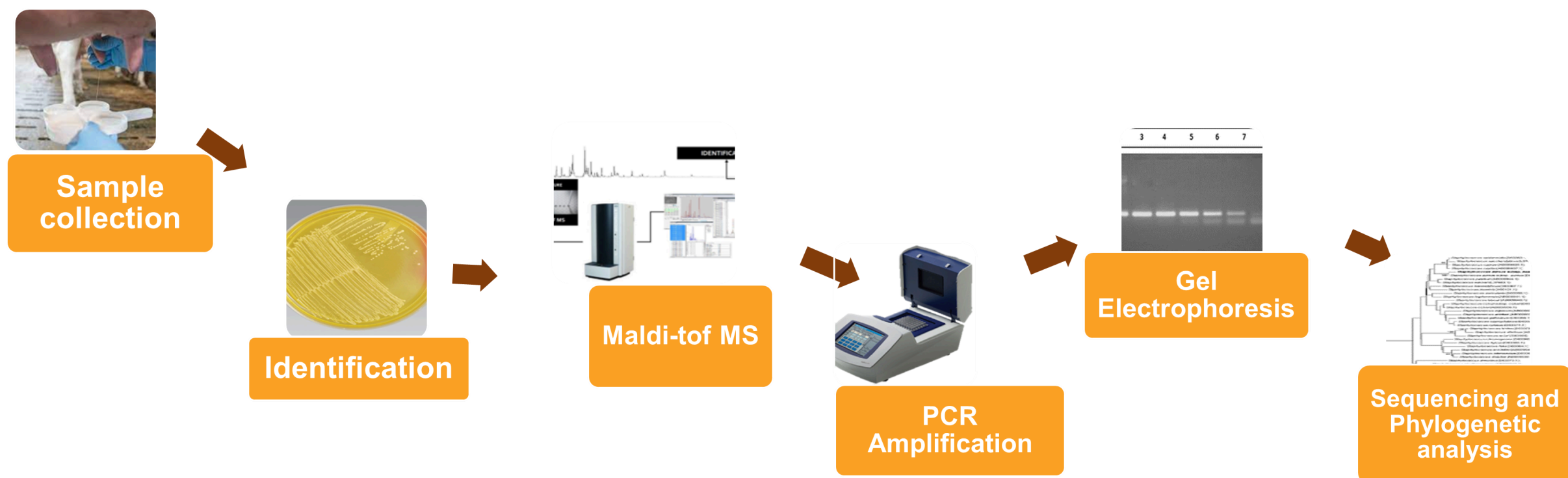
⁴ Unit for Environmental Sciences and Management, North-West University, Potchefstroom, 2531, South Africa

E-mail: nkhasapane@cut.ac.za

1 Background

Mastitis is one of the economically important diseases in the dairy industry. *Staphylococcus* species are amongst the bacteria that cause bovine mastitis (BM) worldwide. These bacteria produce and contain a spectrum of extracellular protein toxins, virulence factors, and antimicrobial resistant properties that are thought to contribute to the pathogenicity of the organism.

3 Workflow



4 Results

- From 166 sampled cows, 33.13% had subclinical mastitis, with a quarter-level prevalence of 54%. Both healthy and mastitis samples showed diverse bacterial species, with five dominant types in each group. In subclinical mastitis cases, species like *P. azotoformans*, *M. bovis*, and *L. lactis* were common. Among 50 bacterial isolates, MALDI-TOF MS identified *S. aureus* (76%) as the most prevalent.
- The 16S rRNA PCR assay also detected *S. aureus* (76%) as the major species.
- Antimicrobial resistance (AMR) tests showed 86% of isolates were resistant to penicillin, 80% to ciprofloxacin, 76% to vancomycin, and 52% to ceftiofur. The *mecA* gene for methicillin resistance was found in 16% of isolates, with various virulence genes present, including *IgG* (52%), *coa* (42%), *spa* (40%), *hla* and *hlb* (38%), *sea* (10%), and *bap* (2%).
- The 38 strains were grouped into seven sequence types, with common AMR genes like *lmrS*, *mepA*, and *tet* identified.

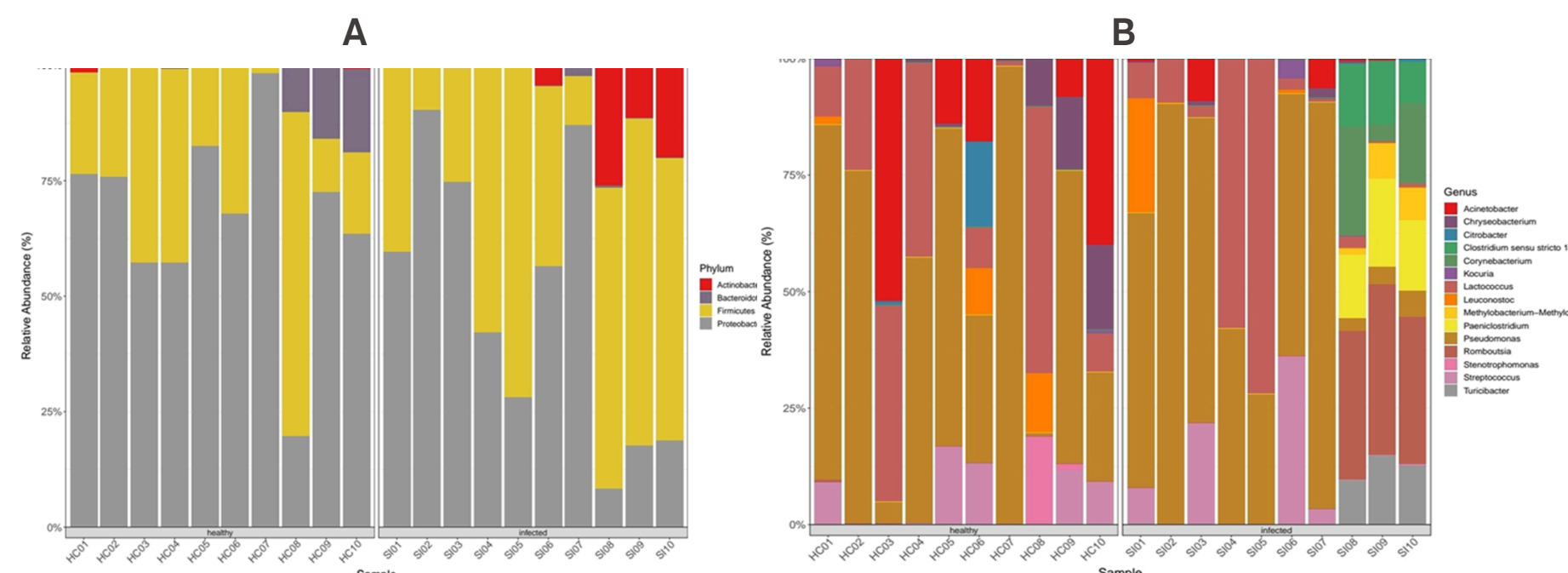


Figure 2 A and B: Distribution of four most abundant phylum (A) and Genus (B) in healthy and infected milk samples. Relative Abundance (%) graphed along the y-axis and sample type along the x-axis

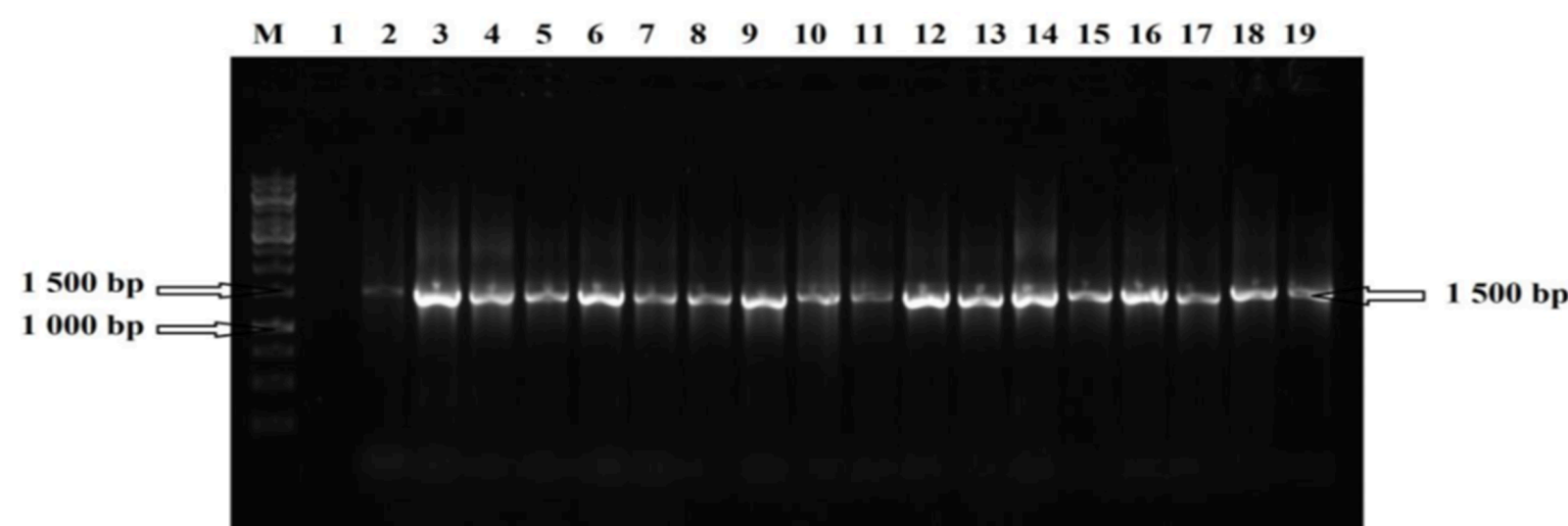


Figure 1: PCR amplified 16S rDNA of *Staphylococcus* strains; molecular weight marker 1kb DNA ladder Lane M; (Lane 1) distilled water, Lane 2-19 (*Staphylococcus* species)

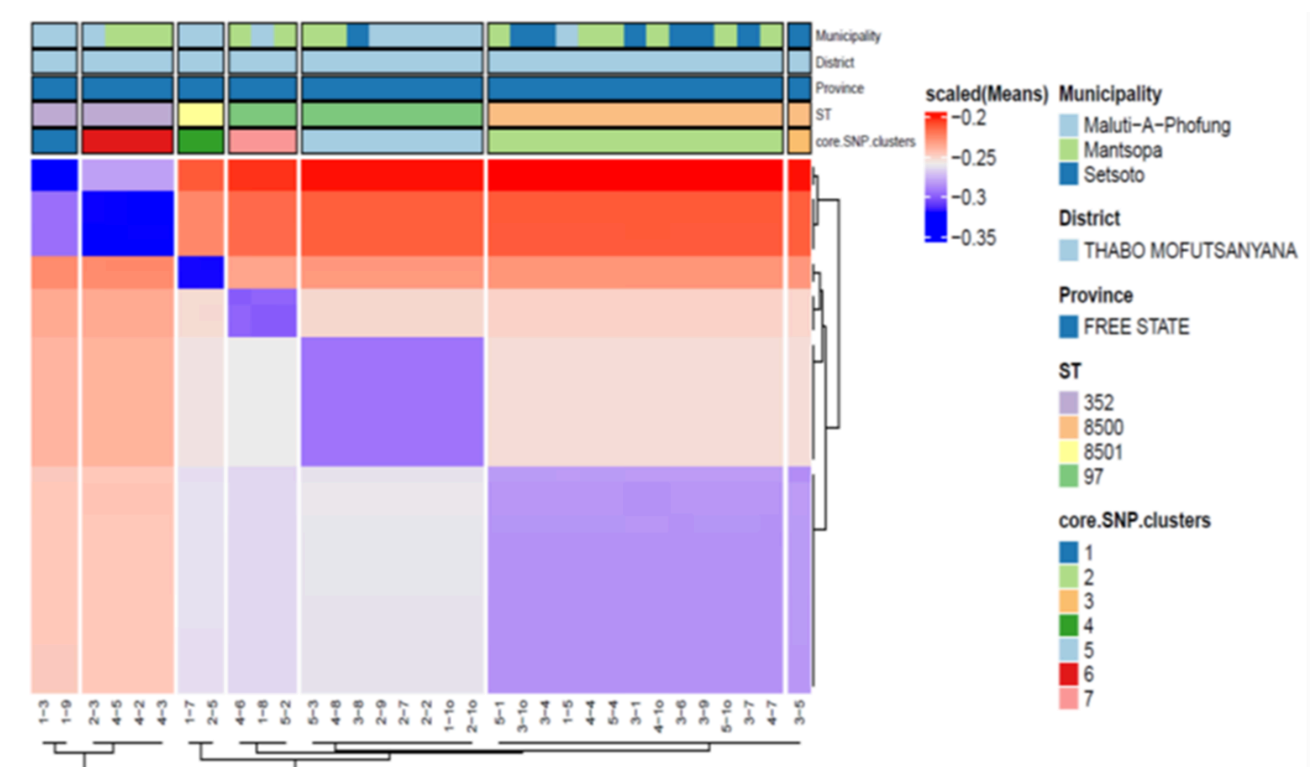


Figure 3: Depicts whole genome single nucleotide polymorphism (wgSNP) clusters, Sequencing types

5 Discussion and Conclusions

- The ability of *S. aureus* to colonize and invade the host may be affected by the combination of *X IgG*, *coa*, *spa*, *hla*, *hnb*, *sea*, and *bap* genes.
- Due to the global presence of these genes, screening *S. aureus* isolates can help predict clinical outcomes and identify dangerous strains.
- This study found a diverse range of *S. aureus* genotypes in dairy cattle in the Free State Province, with genetic differences linked to the geographic origin of the isolates.
- Considering the region and virulence of the strain can help develop strategies to prevent infection spread and implement control measures based on the pathogen and host characteristics.
- Farmers can use information about the circulating strain to decide whether to cull infected animals or isolate positive cows, using sanitary milking practices and an appropriate milking schedule.