Developments in Diagnostics and Antibiotic Resistance of Coagulase Positive, Maltose Negative Staphylococci from Milk of Dairy Cows

Joanne Karzis[a,*], Inge-Mariè Petzer [a], Edward F. Donkin [b], Vinny Naidoo [c], Eric M.C. Etter [a, d]

[a] Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa,

[b] Department of Animal and Wildlife Sciences, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

[c] Department of Research & Postgraduate Studies; Biomedical Research Centre, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

[d] UR AGIRs, Department Environment and Societies – CIRAD, Avenue Agropolis, 34398 Montpellier Cedex 5, France

* Corresponding author. Email: joanne.karzis@up.ac.za

Abstract

In South Africa, as is done with routine Veterinary diagnostics worldwide, focus is on a fast turnaround time and relatively low cost. Staphylococci are identified using the coagulase test (Staphylase test, Oxoid). In more recent years, further diagnosis of coagulase positive staphylococci on maltose agar has been known to differentiate *Staphylococcus aureus* (*S. aureus*) from *Staphylococcus intermedius* group staphylococci (SIG), which comprises of *S. pseudintermedius*, S. *intermedius*, S. *delphini* and others. This became important due to the increase of coagulase positive maltose negative isolates (SIG) in dairy herds in South Africa in recent years, after first being identified in 2006. These organisms was responsible for low somatic cell count (SCC) and showed no antibiotic resistance, unlike the maltose positive *S. aureus.*

The objective of this study was to confirm identification and investigate the seasonal, regional and SCC effects on antibiotic resistance of this emerging pathogen maltose negative *S. aureus*, initially phenotypically identified as potential SIG, of which little is known.

In this study samples identified during routine diagnosis (phenotypic) as potential SIG (coagulase positive, maltose negative staphylococci), were sent for further diagnosis on Matrix Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (Maldi Tof, Bruker) and for genotypic identification using 16s RNA ribosomal sequencing and multi locus sequence typing (MLST) to confirm the diagnosis.

This study analysed retrospective data of 272 of these isolates from milk samples (2009 to 2017). These isolates were tested for antibiotic resistance using the disc diffusion (Kirby Bauer) method for ampicillin, cloxacillin, penicillin G, clindamycin, oxy-tetracycline, cephalexin, cefuroxime, tylosin and cefoxitin, with clinical breakpoints established by CLSI. The samples were from 117 dairy herds, out of the estimated 2000 commercial dairy herds in South Africa. Further testing of 57 of these samples and 56 *S. aureus* (maltose positive) isolates was done using an automated minimum inhibitory concentration (MIC), (Microscan 40 Walkaway system, Beckman Coulter) with the PM32 panels. The susceptible breakpoints for the MIC used were 4 µg/ml and the resistance breakpoints were 32 µg/ml.

The Maldi Tof, 16s sequencing and MLST done confirmed the isolates as a maltose negative strain of *S. aureus* and not part of the SIG group as originally suspected. Predictions of antibiotic resistance for cloxacillin, cefoxitin and cephalexin showed significant differences between SCC categories according to the general linear mixed model (GLMM) ($p<0.05$), but no significant differences for provinces or seasons. Predictions of antibiotic resistance for cephalexin, ampicillin, tylosin and penicillin G showed significant differences according to the GLMM ($p<0.05$) for seasons, but there were no significant differences for provinces or SCC category. The percentage antibiotic resistance of tylosin, ampicillin, oxy-tetracycline, cefalexin & cephalonium to *S. aureus* (maltose negative) increased from 2009 to 2012 where it peaked, for penicillin G and cloxacillin peaked in 2013 and for cefuroxime it peaked in 2011, from the peak for each product the antibiotic resistance (%) decreased until 2017. The MIC results showed resistance mostly to penicillin and or ampicillin for the 57 SIG and 56 maltose positive *S. aureus* (STA) isolates tested.

SIG reacts differently to STA in practice: prevalence per herd – only very small numbers per herd (seems not contagious), no chronic infections were identified and udder parenchyma damage could not be palpated.

Different management and treatment strategies in practice, STA; parlour hygiene, separate and milk last, prognosis: treating, culling; SIG, treat only clinical cases.

This study shows that further differentiation of coagulase positive staphylococci using the maltose test alone is not accurate compared with genotypic identification methods. Strains of *S. aureus* which differ on phenotypic identification, also differ in antibiotic resistance.

Keywords: coagulase positive, maltose negative, *Staphylococcus aureus*, diagnosis, antibiotic resistance,

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