

Diversity and antimicrobial susceptibility profiling of staphylococci isolated from bovine mastitis cases and close human contacts

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ABSTRACT

The objectives of this study were to examine the diversity of *Staphylococcus* spp. recovered from bovine intramammary infections and humans working in close contact with the animals and to evaluate the susceptibility of the staphylococcal isolates to different antimicrobials. A total of 3,387 milk samples and 79 human nasal swabs were collected from 13 sampling sites in the KwaZulu-Natal province of South Africa. In total, 146 *Staph. aureus* isolates and 102 coagulase-negative staphylococci (CNS) were recovered from clinical and subclinical milk samples. *Staphylococcus aureus* was isolated from 12 (15.2%) of the human nasal swabs and 95 representative CNS were recovered for further characterization. The CNS were identified using multiplex-PCR assays, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and *tuf* gene sequencing. Seven *Staphylococcus* spp. were identified amongst the CNS of bovine origin with *Staph. chromogenes* (78.4%) predominating. The predominant CNS species recovered from the human nasal swabs was *Staph. epidermidis*

(80%) followed by *Staph. chromogenes* (6.3%). The antimicrobial susceptibility of all staphylococcal isolates was evaluated using disk diffusion and was supplemented by screening for specific antimicrobial resistance genes. Ninety eight (67.1%) *Staph. aureus* isolates of bovine origin were pansusceptible; 39 (26.7%) isolates were resistant to a single class, and seven (4.8%) isolates were resistant to 2 classes of antimicrobials. Two *Staph. aureus* (1.4%) isolates were multidrug-resistant. Resistance to penicillin was common, with 28.8% of the bovine and 75% of the human *Staph. aureus* isolates exhibiting resistance. A similar observation was made with the CNS, where 37.3% of the bovine and 89.5% of the human isolates were resistant to penicillin. Multidrug-resistance was common amongst the human CNS, with 39% of the isolates exhibiting resistance to 3 or more classes of antimicrobials. The antimicrobial susceptibility results suggest that resistance among staphylococci causing bovine IMI in South Africa is uncommon and not a significant cause for concern. In contrast, antimicrobial resistance was frequently observed in staphylococcal isolates of human origin, highlighting a possible reservoir of resistance genes. Continued monitoring of staphylococcal isolates is warranted to monitor changes in the susceptibility of isolates to different classes of antimicrobials.

Keywords: *Staphylococcus* spp., bovine mastitis, antimicrobial resistance, close human contact

INTRODUCTION

Bovine mastitis, or inflammation of the mammary gland, is one of the most common and economically important diseases affecting the dairy industry worldwide (Barkema et al., 2006). Mastitis primarily occurs in response to microbial infections, although chemical, physical or traumatic factors may also lead to inflammation. A variety of bacteria have been implicated in

bovine intramammary infections IMI (Gentilini et al., 2000), with staphylococci, being considered one of the most significant and prevalent groups (Pyörälä et al., 2011).

Staphylococcus aureus is a contagious udder pathogen that readily spreads between cows at milking (Akineden et al., 2001). The main source of the bacterium is milk from infected quarters, with milk machine teat liners playing a significant role in the transmission of the bacteria among cows and mammary quarters (Zadoks et al., 2002). It is imperative that infected cows are promptly identified so that appropriate control measures can be implemented to curb bacterial transmission (Barkema et al., 2006). Other *Staphylococcus* spp., the so-called coagulase-negative staphylococci (CNS), have traditionally been regarded as opportunistic pathogens of minor importance. This perception has arisen because mastitis caused by CNS is very mild and usually remains subclinical (Tenhagen et al., 2006). However, the significance of the CNS is being reassessed because, in many countries including South Africa, CNS have become the most common bacteria isolated from bovine IMI (Petzer et al., 2009; Taponen and Pyörälä, 2009; Piessens et al., 2011; Sampimon et al., 2011).

At present, the CNS group comprises 51 species and 25 subspecies (LPSN, 2015). In routine mastitis diagnostic laboratories, CNS are usually not identified to species level but are reported as a single group (Taponen et al., 2012). Consequently, limited knowledge is available regarding the epidemiology, pathogenicity and relative importance of the different species in this group. Research emanating primarily from European countries has suggested that some of the CNS species recovered from IMI are specifically adapted to the udder, whereas other species are environmental opportunists that only sporadically cause infections (Taponen, 2008; Thorberg et al., 2009; Piessens et al., 2011). Accurate identification of CNS is paramount in order for the

epidemiology of the different species to be elucidated and appropriate control strategies to be implemented in dairy herds where CNS mastitis is a problem (Sawant et al., 2009). Although the importance of species-level identification of CNS is recognized, the heterogeneity of the species within this group presents challenges in diagnostic laboratories. Biochemical identification of staphylococcal isolates is labor intensive because of the large number of tests required to differentiate the number of species within the genus (Vanderhaeghen et al., 2014). Furthermore, commercial phenotyping systems have been found to have limited accuracy when identifying CNS of veterinary origin, presumably due to the limited number of veterinary isolates incorporated into the databases (Zadoks and Watts, 2009). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used successfully to identify mastitis pathogens (Barreiro et al., 2010; El Behiry et al., 2013); however, the capital costs of the instrumentation limit the use of MALDI-TOF MS to specialized research facilities (Wieser et al., 2012). Molecular methods, such as PCR and sequencing of housekeeping genes, have been shown to have higher discriminatory power, reproducibility and typeability than conventional phenotyping methods and are now being recommended for the accurate identification of CNS (Zadoks and Watts, 2009). Amplification and sequencing of the *tuf* gene, which encodes the elongation factor Tu, has been used successfully to differentiate CNS species (Capurro et al., 2009).

Antimicrobials are commonly used for the treatment of cows with IMI or as a preventative measure during the dry cow period (Sawant et al., 2009). *Staphylococcus aureus* is intrinsically susceptible to most class of antimicrobials but has the extraordinary potential to develop, or acquire, resistance to virtually any antimicrobial to which it is exposed (Chambers and Deleo, 2009). Many of the genes encoding antimicrobial resistance determinants reside on mobile

genetic elements, which can be exchanged between bacteria of the same or different species occupying the same ecological niche (Lindsay and Holden, 2006). This is of great clinical significance, as the transfer of resistance genes can lead to the emergence of more resistant and virulent bacterial strains (Lindsay and Holden, 2006). Although less pathogenic than *Staph. aureus*, CNS often exhibit greater resistance to antimicrobials and also have a greater tendency to develop multidrug resistance (MDR) (Taponen and Pyörälä, 2009). The CNS are believed to serve as reservoirs of antimicrobial resistance genes, which can transfer and integrate into the genome of *Staph. aureus* (Otto, 2013; Vital et al., 2014).

Evaluating the antimicrobial susceptibility of staphylococcal isolates recovered from IMI is important to guide therapeutic treatment of infected animals (Barkema et al., 2006). Moreover, monitoring the antimicrobial resistance of *Staphylococcus* spp. in animals is important from a public health perspective. The close proximity of humans and animals in the dairy environment, presents opportunities for the transmission of bacteria between animal and human hosts (Juhász-Kaszanyitzky et al., 2007). The recent description of livestock-associated methicillin-resistant *Staph. aureus* (LA-MRSA) (Graveland et al., 2011) and *Staph. aureus* strains harboring a novel methicillin-resistance gene, *mecC* (García-Álvarez et al., 2011), have renewed public concerns regarding the role of animals, particularly livestock, as a reservoir and source of antimicrobial resistant bacteria, which can infect humans (Fitzgerald, 2012). Both LA-MRSA and *Staph. aureus* strains harboring the *mecC* gene have been recovered from bovine milk samples in several studies (Vanderhaeghen et al., 2010; Garcia-Álvarez et al., 2011; Unnerstad et al., 2013). Inasmuch as the zoonotic transfer of bacteria is of concern from a human health perspective, the reverse scenario - the anthroponotic transfer of bacteria from humans to animals -

warrants equal consideration (Fitzgerald, 2012). It is therefore important that bacterial populations at the animal-human interface should be monitored.

Antimicrobial-resistant *Staph. aureus*, particularly methicillin-resistant *Staph. aureus* (MRSA), represent a significant public health burden in both hospital and community settings worldwide (Chambers and DeLeo, 2009). The treatment options for infected patients are complicated by the fact that MRSA strains are MDR owing to the carriage of other resistance genes on the cassette chromosome harboring the *mecA* gene (Holmes and Zadoks, 2011). Resistance to frontline antimicrobials such as the glycopeptide vancomycin has been documented and the alarming lack of new antimicrobials is a cause of great concern (Pantosti et al., 2007). Significant efforts are therefore aimed at conserving existing antimicrobials by promoting good antimicrobial stewardship in both human healthcare and veterinary medicine (Morley et al., 2005).

The purpose of this research was to investigate the diversity of *Staphylococcus* spp. responsible for IMI in South African dairy cows and to assess the susceptibility of the different species to antimicrobials commonly used in the veterinary field as well as antimicrobials relevant to human medicine. Concomitantly, individuals working in close contact with the animals were sampled and the diversity and susceptibility profiles of staphylococcal isolates determined and compared with the staphylococcal species of animal origin.

MATERIALS AND METHODS

Sample collection and processing

Ethical clearance for this investigation was obtained from the Animal Ethics Committee, Faculty of Veterinary Science, University of Pretoria (H010-13) and the University of Pretoria, Faculty of Health Sciences Research Ethics Committee (No.295/2013).

KwaZulu-Natal (KZN) is one of the nine provinces in the Republic of South Africa. Commercial dairy herds (n = 9; identified by the letters A, B, C, E, F, G, I, J and L), communal animal herds (n = 2; collectively identified by the letter M), and agriculture college herds (n = 2; identified by the letters D and H) in KZN were recruited to participate in this study through private and state veterinarians in the province. Participation was voluntary. For commercial dairy herds, the sole criterion for inclusion in this study was a history of staphylococcal mastitis. Because of the limited number of agriculture college herds in the province and the limited accessibility to communal animals, no specific selection criteria could be implemented. Herd sizes ranged from 95 to 1,450 (commercial herds), 40 to 50 (agriculture college herds), and 3 to 8 (communal animals). A single sampling was carried out at each site between October 2013 and March 2014.

Milk samples were aseptically collected into sterile sample tubes following cleaning and disinfection of the teat ends and stripping of the first 2 to 3 squirts of milk. At each of the sampling sites, close contact workers including, animal owners, dairy laborers and veterinary practitioners were approached and asked to participate in the study by providing a self-collected nasal swab (Copan, Brescia, Italy). Following collection, all milk samples and nasal swab specimens were packed on ice and transported to the laboratory.

All milk samples were visually inspected following receipt in the laboratory. Milk samples showing any visual evidence of abnormality, such as the presence of clots, flakes or blood, were classed as being clinical. No SCC was performed on clinical samples. All milk samples with a normal appearance were analyzed by flow cytometry using a Somacount instrument (Bentley Instruments, Chaska, MN) and the number of somatic cells per milliliter of sample was determined.

Microbiological analysis of milk samples and nasal specimens

Ten microliters of each milk sample was inoculated onto one quadrant of a Columbia blood agar plate (Oxoid, Basingstoke, UK) supplemented with 5% ovine blood. Culture plates were incubated (NuAire, Plymouth, MN) at 37°C for 48 h. Nasal specimens were transferred to brain heart infusion broth (Oxoid) and incubated at 37°C overnight before being streaked onto Columbia blood agar (Oxoid) supplemented with 5% ovine blood, BBL Columbia nalidixic acid agar (Becton Dickinson, Sparks, MD) and oxacillin-resistance screening agar base (Oxoid). All plates were incubated at 37°C for 48 h before being examined for the presence of staphylococcal-like colonies (Quinn et al., 1999). Preliminary testing of staphylococcal isolates included Gram-staining and testing for catalase production. All catalase-positive, gram-positive coccus-shaped bacterial isolates were tentatively identified as *Staphylococcus* spp. and tested for coagulase-production using rabbit plasma (Bio-Rad, Hercules, CA) and clumping factor using a commercial latex agglutination kit (Pastorex StaphPlus kit, Bio-Rad) (Quinn et al., 1999).

Only staphylococcal isolates recovered from milk samples with a somatic cell count $\geq 200,000$ cells/mL were selected for further characterization. Additional selection criteria for CNS

included the occurrence of isolates in pure growth and the presence of 5 or more colonies (≥ 500 cfu/ml milk) on primary culture. When more than one type of CNS was isolated from different quarters of the same cow, based on phenotypic appearance, both isolates were retained for further testing. With respect to the staphylococcal isolates recovered from the human nasal swabs, all *Staph. aureus* isolates were retained along with representative CNS colonies from each individual. All isolates were stored in 25% glycerol (Merck, Darmstadt, Germany) at -80°C (NuAire) until required.

Identification of staphylococci and screening for mecA and pvl genes using PCR

One or two bacterial colonies were suspended in 50 μL sterile deionized water. Bacterial DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. All staphylococcal isolates were amplified using a multiplex PCR assay (multiplex 1) to confirm genus identification, to identify *Staph. aureus* isolates and to simultaneously screen isolates for the methicillin resistance gene, *mecA*, and the Pantan-Valentine leukocidin encoding gene, *pvl*. Each PCR assay was carried out in a 25 μL volume comprising of 1X PCR buffer (Promega, Wisconsin, USA), 0.3 mM dNTP mixture (Promega, Wisconsin, USA), 3 mM MgCl_2 (Promega, Wisconsin, USA), 0.2 μM of each primer, 1.5 U HotStart GoTaq (Promega, Wisconsin, USA) and 2 μL of DNA extract. Reactions were cycled in a MJ Mini thermocycler (BioRad, Chaska, MN) with the following parameters: initial denaturation (95°C for 2 min) followed by 30 cycles of amplification (94°C for 45 s, 51°C for 90 s and 72°C for 90 s) ending with a final extension step at 72°C for 10 min. A positive control and negative control (deionized water) was set up with every batch of PCR samples amplified. Details of all oligonucleotide primers and PCR controls are summarized in Table 1. The PCR products were resolved in a 1.5% agarose gel (SeaKem, Lonza, Rockland, ME), in 1X Tris-

Table 1. Details of PCR primers used for the identification and characterization of *Staphylococcus* isolates

Gene target	Primer Sequence (5' – 3') ¹	Size of PCR product (bp)	Multiplex assay	Positive control	Reference
16S rRNA	AACTCTGTTATTAGGGAAGAACA CCACCTTCTCCGTTTGTCCAC	756	1, 2, 3, 4, 5		McClure et al. (2006)
<i>nuc</i>	TCGCTTGCTATGATTCTGG GCCAATGTTCTACCATAGC	359	1	<i>S. aureus</i> ATCC 25923	Sasaki et al. (2010)
<i>luk S/F</i>	ATCATTAGGTAAAATGCTCTGGACATGATCCA GCATCAAGTGTATTGGATAGCAAAAAGC	433	1	<i>S. aureus</i> ²	McClure et al. (2006)
<i>mecA</i>	GTAGAAATGACTGAACGTCCGATAA CCAATTCCACATTGTTTCGGTCTAA	310	1	<i>S. aureus</i> ²	McClure et al. (2006)
<i>S. chromogenes</i> <i>sodA</i>	GCGTACCAGAAGATAAAACAACCTC CATTATTTACAACGAGCCATGC	222	2	<i>S. chromogenes</i> ²	Shome et al. (2011)
<i>S. haemolyticus</i> <i>groESL</i>	GGTCGCTTAGTCGGAAACAAT CACGAGCAATCTCATCACCT	271	2, 3	<i>S. haemolyticus</i> ATCC 29970	Chiang et al. (2012)
<i>S. xylosus</i> <i>groESL</i>	CGCCATATTACTTATACCTCC AAGGCGATAATGTAGTATTCA	180	2	<i>S. xylosus</i> ATCC 29971	Chiang et al. (2012)
<i>S. simulans</i> <i>gap</i>	AGCTTCGTTACTTCTTCGATTTGT AAAAGCACAAAGCTCACATTGAC	472	2	<i>S. simulans</i> ATCC 27851	Shome et al. (2011)
<i>S. epidermidis</i> <i>rdr</i>	AAGAGCGTGGAGAAAAGTATCAAG TCGATACCATCAAAAAGTTGG	130	2, 3	<i>S. epidermidis</i> ATCC 12228	Shome et al. (2011)
<i>S. warneri</i> <i>nuc</i>	CGTTTGTAGCAAAAACAGGGC GCAACGAGTAACCTTGCCAC	999	2	<i>S. warneri</i> ATCC 49454	Hirota et al. (2011)
<i>S. hominis</i> <i>nuc</i>	TACAGGGCCATTTAAAGACG GTTTCTGGTGTATCAACACC	177	3	<i>S. hominis</i> ²	Hirota et al. (2011)
<i>S. capitis</i> <i>nuc</i>	ACTACGCCTATGATTATTGC GAYGCTTCTTTACCATAGGG	525	3	<i>S. capitis</i> ATCC 35661	Hirota et al. (2011)
<i>tuf</i>	GCCAGTTGAGGACGTATTCT CCATTTCAGTACCTTCTGGTAA	412	Uniplex	None	Heikens et al. (2005)
<i>blaZ</i>	AAGAGATTTGCCTATGCCTC GCTTGACCACTTTTATCAGC	517	4	<i>S. aureus</i> ATCC 29213	Vesterholm-Nielsen et al. (1999)
<i>mecC</i>	GA190AAAAAAGGCTTAGAACGCCTC GAAGATCTTTCCGTTTTCAGC	138	4	<i>S. aureus</i> ²	Stegger et al. (2011)
<i>vanA</i>	CATGAATAGAATAAAAAGTTGCAATA CCCCTTTAACGCTAATACGATCAA	1,030	4	<i>E. faecalis</i> ATCC 700221	Clark et al. (1993)
<i>vanB</i>	GTGACAAAACCGGAGCGAGGA CCGCCATCCTCCTGCAAAAAA	433	4	<i>E. faecalis</i> ATCC 51299	Clark et al. (1993)
<i>tetM</i>	AGTGGAGCGATTACAGAA CATAATGCTCTGGCGTGTCTA	158	5	<i>S. aureus</i> ²	Strommenger et al. (2003)
<i>tetK</i>	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360	5	<i>S. aureus</i> ²	Strommenger et al. (2003)
<i>aacA-aphD</i>	TAATCCAAGGACAATAAGGGC GCCACACTATCATAACCCTA	227	5	<i>S. aureus</i> ²	Strommenger et al. (2003)

¹All primers were synthesized by Inqaba Biotechnical Industries (Pretoria, South Africa).

²Verified field isolate: clavulanic acid; FOX = ceftiofur; KF = cephalothin; TET = tetracycline; SXT = trimethoprim and sulfamethoxazole; MXF = moxifloxacin; DA = clindamycin; E = erythromycin; GEN = gentamicin; S = streptomycin; FD = fusidic acid; LZD = linezolid; CPT = ceftaroline

borate-EDTA (TBE) buffer (Melford, Ipswich, UK). Following staining in a 1 µg/mL ethidium bromide solution (BioRad) the PCR products were visualized and documented (UVIPro, Uvitec, Cambridge, UK).

Speciation of coagulase-negative staphylococci using PCR, MALDI-TOF MS and tuf gene sequencing

Following genus-specific identification, CNS were identified to species level using a combination of methods. All test isolates were amplified using 1 of 2 multiplex PCR assays (multiplex 2 and 3) based on the origin of the isolates. Reactions were prepared in 25-µL volumes using the Qiagen Multiplex PCR kit and amplified according to the manufacturer's instructions. The PCR products were analyzed as described above with the exception that a 1.8% agarose gel (SeaKem, Lonza) was used to resolve the PCR amplicons.

With the exception of one bovine CNS isolate that failed to grow, overnight cultures of all isolates were preserved in chromatography-grade ethanol (Merck) and submitted for MALDI-TOF MS analysis (Bruker Daltonics GmbH, Bremen, Germany). Coagulase-negative staphylococcal isolates that could not be identified using the multiplex-PCR assay or MALDI-TOF MS or where discordant species identifications were obtained using the 2 methods were analyzed further using *tuf* gene sequencing. The *tuf* gene was amplified according to the method described by Heikens and coworkers (2005), and PCR amplicons were submitted to Inqaba Biotechnical Industries (Pretoria, South Africa) for sequencing. Consensus sequences were analyzed in GenBank and cut-off values for the percentage of identity were adjusted to $\geq 97\%$ sequence similarity.

Antimicrobial susceptibility testing of staphylococcal isolates

The antimicrobial susceptibility of the staphylococcal test isolates to 15 antimicrobials was carried out using the agar disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2008). The antibiotics tested (Oxoid) included amoxicillin and clavulanate (20 µg/10 µg), ampicillin (10 µg), cefoxitin (30 µg), ceftaroline (30 µg), cephalothin (30 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), moxifloxacin (5 µg), penicillin (10 U), streptomycin (10 µg), tetracycline (30 µg) and trimethoprim/sulfamethoxazole (1.25/23.75 µg). The clindamycin and erythromycin disks were placed within 25 mm of each other on the agar surface in order to facilitate the detection of inducible clindamycin resistance or the “D-effect” (Fiebelkorn et al., 2003). A control isolate, *Staph. aureus* ATCC 25923, was tested together with each batch of field isolates to ensure the validity of the test results. The zone measurements for all antimicrobials, with the exception of fusidic acid and streptomycin, were compared with CLSI breakpoints based on data derived from human medicine (CLSI, 2008, 2014). In the case of fusidic acid, the breakpoint recommended by the European committee on Antimicrobial Susceptibility Testing (EUCAST, 2014) was used and for streptomycin a breakpoint of ≥ 14 mm was used as described by Kim and coworkers (2004) and Shittu and Lin (2006). The production of β -lactamase by *Staph. aureus* isolates was determined by examining the edge of the zone of inhibition surrounding the penicillin disk. Isolates showing abrupt, heaped edges were recorded as being resistant to penicillin, irrespective of the zone diameter (CLSI, 2014). Beta-lactamase production by CNS isolates was determined using nitrocefin-impregnated sticks (Oxoid) in accordance with the manufacturer’s instructions.

Resistance categories were defined based on recommendations made by Schwarz and coworkers (2010). Briefly, isolates showing susceptibility to all classes of antimicrobials tested were termed

pansusceptible; isolates showing resistance to 1 or 2 classes of antimicrobials were termed resistant and isolates showing resistance to 3 or more classes of antimicrobials were termed multidrug-resistant (MDR).

PCR screening of staphylococcal isolates for antimicrobial resistance determinants

Staphylococcal isolates were screened for a combination of 7 antimicrobial resistance genes using 2 multiplex-PCR assays. Reactions were prepared in 25- μ L volumes using the Qiagen Multiplex PCR kit and included primers specific for the *blaZ*, *mecC*, *vanA* and *vanB* (multiplex 4) and *tetK*, *tetM* and *aacA-aphD* (multiplex 5) genes. Amplification of PCR reactions was carried out in a MJ Mini thermocycler (BioRad) using the thermocycling conditions recommended by the kit manufacturer. Electrophoresis was carried out as described above.

RESULTS

Microbiological analysis

A total of 3,387 milk samples, from 1,374 cows, and 79 human nasal swab specimens were collected from 13 dairy herds. One hundred and forty-six *Staph. aureus* and 102 CNS isolates cultured from the milk samples fulfilled the selection criteria and were retained for further analysis. *Staphylococcus aureus* was recovered from 12 (15.2%) of the human nasal swabs. All 12 *Staph. aureus* isolates and the 95 CNS isolates recovered from the nasal swabs were retained for analysis.

Identification of staphylococci and screening for mecA and pvl genes by PCR

All of the analyzed staphylococcal isolates were genotypically identified as *Staphylococcus* spp. using the first multiplex-PCR assay. Furthermore, all of the isolates phenotypically identified as *Staph. aureus* were confirmed to be *Staph. aureus* using the same multiplex PCR assay. None of the bovine or human staphylococcal isolates was positive for the Panton-Valentine leucocidin-encoding gene, *pvl*. Fifteen CNS of human origin tested positive for the *mecA* gene.

Speciation of coagulase-negative staphylococci using PCR, MALDI-TOF MS and tuf gene sequencing

A combination of 2 multiplex PCR assays and MALDI-TOF MS was used to identify the 102 CNS isolates of bovine origin and 95 CNS isolates of human origin. Five bovine isolates originating from 2 different farms were not identified using either the multiplex PCR assay or MALDI-TOF MS and were analyzed further using *tuf* gene sequencing. The consensus *tuf* DNA sequences of the 5 isolates showed 99% to 100% agreement with sequences already deposited in Genbank (Acc No. EU571021.1). The sequences corresponded to an unidentified *Staphylococcus* species recovered from bovine mastitis cases deposited by Capurro and coworkers (2009).

A further 9 CNS were analyzed using *tuf* gene sequencing in order to confirm the species identification obtained using MALDI-TOF MS. The percentage distribution of coagulase-negative *Staphylococcus* species isolated from both the milk samples and human specimens is presented in Table 2. Seven different *Staphylococcus* spp. were identified among the CNS isolates of bovine origin, with the majority of the isolates being identified as *Staph. chromogenes* (78.4%). Other species identified included *Staph. xylosus* (4.9%), *Staph. hyicus* (3.9%), *Staph.*

Table 2. Phenotypic resistance (no.; % in parentheses) of bovine and human CNS to the different antimicrobials evaluated

Origin of isolates	Staphylococcus species identification	Total no. of isolates (%)	No. of MDR ¹ isolates (%)	Antimicrobial evaluated ²													
				PEN	AMP	AMC	FOX	KF	TET	SXT	MXF	DA	E	CN	S	FD	LZD
BOVINE	<i>S. chromogenes</i>	80 (78.4)	2 (2.5)	33 (41.3)	33 (41.3)	0	0	0	6 (7.5)	0	0	0	0	0	4 (5.0)	0	0
	<i>S. epidermidis</i>	1 (0.9)	0	1 (100)	1 (100)	0	0	0	0	1 (100)	0	0	0	0	0	0	0
	<i>S. haemolyticus</i>	2 (1.9)	0	1 (50)	1 (50)	0	0	0	0	0	0	0	0	0	0	0	0
	<i>S. hyicus</i>	4 (3.9)	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (25)	0
	<i>S. lugdunensis</i>	1 (0.9)	0	1 (100)	1 (100)	0	0	0	0	0	0	0	0	0	0	0	0
	<i>S. simulans</i>	4 (3.9)	0	0	0	0	0	0	1 (25)	0	0	0	0	0	1 (25)	0	0
	<i>S. xylosum</i>	5 (4.9)	0	0	0	0	0	0	2 (40)	0	0	0	0	0	0	1 (20)	0
	<i>Staphylococcus spp.</i>	5 (4.9)	0	2 (40)	2 (40)	0	0	0	0	0	0	0	0	0	0	0	0
	Total	102 (100)	2 (1.9)	38 (37.3)	37 (36.3)	0	0	0	9 (8.8)	1 (1)	0	0	0	0	5 (4.9)	2 (2)	0
HUMAN	<i>S. capitis</i>	1 (1.1)	0	1 (100)	1 (100)	0	0	0	1 (100)	0	0	0	0	0	0	0	0
	<i>S. chromogenes</i>	6 (6.3)	1 (16.7)	5 (83.3)	5 (83.3)	0	0	0	0	0	0	1 (16.7)	1 (16.7)	0	1 (16.7)	0	0
	<i>S. epidermidis</i>	76 (80)	29 (38.2)	71 (93.4)	71 (93.4)	4 (5.3)	12 (15.8)	0	51 (67.1)	30 (39.5)	3 (3.9)	6 (7.9)	15 (19.7)	3 (3.9)	1 (1.3)	0	0
	<i>S. haemolyticus</i>	5 (5.3)	5 (100)	5 (100)	5 (100)	0	5 (100)	0	5 (100)	5 (100)	4 (80)	0	2 (40)	0	0	0	0
	<i>S. hominis</i>	1 (1.1)	1 (100)	1 (100)	1 (100)	0	0	0	1 (100)	0	0	0	1 (100)	0	0	0	0
	<i>S. lugdunensis</i>	1 (1.1)	0	1 (100)	1 (100)	0	0	0	1 (100)	0	0	0	0	0	0	0	0
	<i>S. pseudintermedius</i>	1 (1.1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>S. sciuri</i>	2 (2.1)	1 (50%)	1 (50)	0	0	0	0	0	0	0	2 (100)	0	0	0	1 (50)	0
	<i>S. simulans</i>	2 (2.1)	0	0	0	0	0	0	2 (100)	0	0	0	0	0	0	0	0
	Total	95 (100)	37 (39.0)	85 (89.5)	84 (88.4)	4 (4.2)	17 (17.9)	0	61 (64.2)	35 (36.8)	7 (7.4)	9 (9.5)	19 (20.0)	3 (3.2)	2 (2.1)	1 (1.1)	0

¹ Multidrug resistance (resistance to ≥ 3 antimicrobial classes).

² PEN = penicillin; AMP = ampicillin; AMC = amoxicillin/clavulanic acid; FOX = ceftiofur; KF = cephalothin; TET = tetracycline; SXT = trimethoprim and sulfamethoxazole; MXF = moxifloxacin; DA = clindamycin; E = erythromycin; GEN = gentamicin; S = streptomycin; FD = fusidic acid; LZD = linezolid

simulans (3.9%), *Staph. haemolyticus* (1.9%), *Staph. lugdunensis* (0.9%) and *Staph. epidermidis* (0.9%). Nine species were identified amongst the CNS recovered from the human specimens with *Staph. epidermidis* (80%), *Staph. chromogenes* (6.3%) and *Staph. haemolyticus* (5.3%) predominating.

Antimicrobial susceptibility testing of staphylococcal isolates

All staphylococcal isolates were evaluated for susceptibility to antimicrobials used for the treatment of bovine IMI as well as antimicrobials relevant to human medicine. The antimicrobial susceptibility profiles of the *Staph. aureus* isolates of bovine and human origin are summarized in Table 3. Isolates showing intermediate susceptibility were grouped together with isolates showing resistance to the antimicrobial evaluated.

The *Staph. aureus* isolates of bovine origin were divided into 8 antibiotypes based on their susceptibility patterns to the different classes of antimicrobials evaluated. Ninety eight (67.1%) *Staph. aureus* isolates of bovine origin were susceptible to all of the antimicrobial classes evaluated (pansusceptible). Thirty nine (26.7%) isolates were resistant to a single class of antimicrobials, whereas 7 (4.8%) were resistant to 2 classes. Two (1.4%) *Staph. aureus* isolates, from different herds, exhibited MDR. One of these isolates was resistant to the β -lactams penicillin and ampicillin, as well as tetracycline and potentiated sulphonamides. The second MDR isolate showed resistance to penicillin and ampicillin, tetracycline and streptomycin. The *Staph. aureus* isolates of human origin were divided into 4 antibiotypes. Three (25%) of the 12 *Staph. aureus* isolates were pansusceptible; 4 *Staph. aureus* isolates (33.3%) were resistant to a single class of antimicrobials; and a further 4 isolates (33.3%) were resistant to 2 classes of antimicrobials. A single *Staph. aureus* isolate was found to be MDR, showing resistance to

Table 3. Phenotypic susceptibility¹ of *Staphylococcus aureus* isolates recovered from bovine and human hosts

Origin of isolates	Farm of origin (no. of isolates)	No. (%) of isolates with phenotypic pattern	No. of classes of antimicrobials to which resistance was detected	Antimicrobial evaluated ²														
				PEN	AMP	AMC	FOX	KF	TET	SXT	MXF	DA	E	GEN	S	FD	LZD	CPT
BOVINE	A(3) C(3) D(15) E(28) F(20) G(9) H(3) I(4) L(13)	98 (67.1)	0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	A(1)	1 (0.7)	1	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
	A(1) E(1) F(2)	4 (2.7)	1	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
	B(13) G(7) I(14)	34 (23.3)	1	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S
	B(1) I(3) L(1) G(1)	6 (4.1)	2	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S
	L(1)	1 (0.7)	2	S	S	S	S	S	R	S	S	S	S	S	R	S	S	S
	G(1)	1 (0.7)	3	R	R	S	S	S	R	S	S	S	S	S	R	S	S	S
	L(1)	1 (0.7)	3	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S
	No. Isolates ²	146 (100)		42 (28.8)	42 (28.8)	0	0	0	4 (2.7)	1 (0.7)	0	0	0	0	12 (8.2)	0	0	0
HUMAN	D(1) E(1) M(1)	3 (25)	0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	A(1) B(1) C(1) J(1)	4 (33.3)	1	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S
	B(1) F(1) J(2)	4 (33.3)	2	R	R	S	S	S	R	S	S	S	S	S	S	S	S	S
	L(1)	1 (8.3)	3	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S
	No. isolates ²	12 (100)		9 (75)	9 (75)	0	0	0	5 (41.6)	1 (8.3)	0	0	0	0	0	0	0	0

¹ S = susceptible; R = resistant

² PEN = penicillin; AMP = ampicillin; AMC = amoxicillin

penicillin and ampicillin, as well as tetracycline and the potentiated sulphonamides. None of the *Staph. aureus* isolates of bovine or human origin showed resistance to cefoxitin (surrogate for methicillin and other penicillinase-resistant β -lactams), ceftaroline, cephalothin, clindamycin, erythromycin, fusidic acid, gentamicin, linezolid or moxifloacin.

The antimicrobial susceptibility of the different CNS species of bovine and human origin are summarized in Tables 2 and 4. Phenotypic resistance to penicillin and ampicillin was observed in 37.3% of the bovine CNS isolates and 89.5% of the human CNS isolates respectively.

Amongst the 102 bovine CNS isolates evaluated, only two *Staph. chromogenes* (1.9%) isolates showed MDR. None of the bovine CNS isolates showed phenotypic resistance to cefoxitin, cephalothin, clindamycin, erythromycin, gentamicin, linezolid, or moxifloxacin. By comparison, 37 (39%) of the human CNS isolates were MDR. The resistance profiles of the MDR isolates are detailed in Table 4. None of the CNS isolates of human origin showed phenotypic resistance to cephalothin or linezolid. The 15 human CNS isolates, which tested positive for the presence of the *mecA* gene using the first multiplex-PCR assay, and were subsequently identified as *Staph. epidermidis* (n = 12) and *Staph. haemolyticus* (n = 5), were found to be phenotypically resistant to cefoxitin.

PCR screening of staphylococcal isolates for antimicrobial resistance determinants

The *blaZ* gene was detected in 172 of the 355 (48.5%) staphylococcal isolates examined. All 172 isolates had been found to be resistant to penicillin when tested phenotypically. Two isolates, 1 *Staph. sciuri* (human origin) and 1 *Staph. chromogenes* (bovine origin), that were found to be phenotypically resistant to penicillin did not test positive for the *blaZ* gene using the multiplex PCR assay. None of the staphylococcal isolates tested positive for the presence of the *tetM* gene.

Table 4. Antimicrobial profiles of CNS isolates showing multiple drug resistance¹

Origin of isolates	Species	Farm of origin ²	Number of classes of antimicrobials resistance detected	Antimicrobial evaluated ³														
				PEN	AMP	AMC	FOX	KF	TET	SXT	MXF	DA	E	CN	S	FD	LZD	
BOVINE	<i>S. chromogenes</i>	D(2)	3	R	R	S	S	S	R	S	S	S	S	S	R	S	S	
	<i>S. chromogenes</i>	F(1)	4	R	R	S	S	S	S	S	S	R	R	S	R	S	S	
HUMAN	<i>S. epidermidis</i>	A(1)	2	R	R	S	R	S	R	S	S	S	S	S	S	S	S	
		A(1) C(3) D(1) J(1) L(1) M(1)	3	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S
		A(1) J(1)	3	R	R	S	R	S	R	S	S	S	R	S	S	S	S	S
		A(1)	3	R	R	S	S	S	S	R	S	S	R	S	S	S	S	S
		A(1)	3	R	R	S	S	S	S	R	S	S	S	R	S	S	S	S
		E(1)	3	R	R	S	R	S	R	S	S	S	S	S	S	R	S	S
		F(1)	3	R	R	S	S	S	R	S	R	S	S	S	S	S	S	S
		H(1)	3	R	R	R	S	S	R	R	S	S	S	S	S	S	S	S
		J(1)	3	R	R	S	R	S	R	R	S	S	S	S	S	S	S	S
		A(1)	4	R	R	S	S	S	R	R	R	S	S	R	S	S	S	S
		A(1) C(1)	4	R	R	S	S	S	R	S	S	R	R	S	S	S	S	S
		E(1) J(1)	4	R	R	S	R	S	R	R	S	S	R	S	S	S	S	S
		E(1)	4	R	R	S	S	S	S	R	S	R	R	S	S	S	S	S
		E(1)	4	R	R	S	S	S	R	S	S	R	R	S	S	S	S	S
		I(1)	4	R	R	R	R	S	S	R	R	S	S	R	S	S	S	S
		J(1)	4	R	R	S	R	S	R	R	S	S	R	S	S	S	S	S
		J(1)	5	R	R	S	R	S	R	R	R	S	R	R	S	S	S	S
		J(1)	5	R	R	S	S	S	R	R	S	R	R	S	S	S	S	S
	H(1)	6	R	R	S	R	S	R	R	R	R	S	R	R	S	S	S	
	<i>S. haemolyticus</i>	A(1) H(1) L(1)	4	R	R	S	R	S	R	R	R	S	S	S	S	S	S	
		H(1)	4	R	R	S	R	S	R	R	S	S	R	S	S	S	S	
		M(1)	5	R	R	S	R	S	R	R	R	S	R	S	S	S	S	
	<i>S. hominis</i>	C(1)	3	R	R	S	S	S	R	S	S	S	R	S	S	S	S	
<i>S. sciuri</i>	E(1)	3	R	R	S	S	S	S	S	S	S	R	S	S	S	R		

¹ S = sensitive; R = resistant

² Number of isolates in parentheses

³ PEN = penicillin; AMP = ampicillin; AMC = amoxicillin/clavulanic acid; FOX = ceftiofur; KF = cephalothin; TET = tetracycline; SXT = trimethoprim and sulfamethoxazole; MXF = moxifloxacin; DA = clindamycin; E = erythromycin; GEN = gentamicin; S = streptomycin; FD = fusidic acid; LZD = linezolid;

The *tetK* gene was detected in 76 of the 79 (96.2%) isolates which were phenotypically resistant to tetracycline. Three of the staphylococcal isolates that were phenotypically resistant to tetracycline did not carry either the *tetK* or *tetM* genes. Nine isolates of human origin (4 *Staph. epidermidis*, 5 *Staph. haemolyticus*) tested positive for the presence of the aminoglycoside resistance determinant *aacA-aphD*. None of the bovine isolates were found to be carrying this specific gene. None of the staphylococcal isolates were positive for the *mecC* gene or the vancomycin resistance determinants *vanA* and *vanB*.

DISCUSSION

Staphylococci are a common cause of bovine IMI worldwide, including South Africa (Petzer et al., 2009). This study was not designed to carry out a prevalence study but rather to facilitate the collection of a representative number of staphylococcal isolates from different dairy operations in KZN for further characterization. Consequently, the selection of participating commercial dairy herds was based on the criterion that each of the herds had a history of IMI caused by *Staphylococcus* spp.

A combination of methods was used to identify the CNS isolates of bovine and human origin. This approach was used in order to improve the accuracy of bacterial identification at the species level. The predominant CNS species recovered from the milk samples analyzed in this study was *Staph. chromogenes*, which accounted for 78.4% of the isolates examined. The proportion of different *Staphylococcus* species recovered from bovine IMI has varied between studies carried out in different countries. In general, *Staph. chromogenes*, *Staph. haemolyticus*, *Staph. epidermidis*, *Staph. simulans* and *Staph. xylosus* are usually among the most commonly isolated

species (Vanderhaegen et al., 2015). In Sweden, Persson-Waller and coworkers (2011) found *Staph. chromogenes* (24%) and *Staph. epidermidis* (22%) to be the most common CNS species identified amongst a collection of isolates assembled from 2 national mastitis surveys. A study in Switzerland reported *Staph. xylosus* (36.0%) and *Staph. chromogenes* (16.8%) to be among the most common CNS causing mastitis (Frey et al., 2013). In the United States, Park and coworkers (2011) identified 11 CNS species from cases of mastitis, with *Staph. chromogenes* (72.2%), *Staph. xylosus* (9.1%), and *Staph. haemolyticus* (6.1%) being the most common species identified. It has been suggested that the distribution of CNS species causing IMI is herd-specific and may be influenced by specific management practices that may vary between countries (Thorberg et al., 2009; Supré et al., 2011). Species-level epidemiological studies conducted in European dairy herds have shown that *Staph. chromogenes* is a more specific udder pathogen than other CNS and tends to cause persistent IMI (Supré et al., 2011). *Staphylococcus chromogenes* was also found to have a significant effect on the SCC of infected quarters, comparable to counts induced by *Staph. aureus* infections (Supré et al., 2011).

Five of the CNS isolates remained unidentified following PCR, MALDI-TOF MS and sequencing of the *tuf* gene. All 5 of the isolates had the same *tuf* gene sequence and in all likelihood are the same species. The *tuf* gene sequence showed 99% to 100% similarity with sequence data deposited by Capurro and coworkers (2009), which interestingly belonged to an isolate recovered from mastitic milk samples in Finland. In recent years, several new *Staphylococcus* species have been described. Supré and coworkers (2010) described a novel *Staphylococcus* species, *Staph. devriesei*, which had been isolated from bovine teat apices as well as milk from study herds in Belgium and The Netherlands. Taponen and coworkers (2012) published details describing a new, previously unidentified *Staphylococcus* species, named

Staph. agnetis. Isolates representing this newly described species were recovered from bovine milk samples (Taponen et al., 2012). Further genotyping studies will be undertaken to identify the unidentified *Staphylococcus* spp. recovered from the milk samples analyzed in the current study.

The predominant CNS recovered from the human nasal swab samples was *Staph. epidermidis*. This is not surprising as *Staph. epidermidis* is a known commensal forming part of the natural microflora on human skin (Thorberg et al., 2006). Molecular typing studies conducted by Thorberg and coworkers (2006) in Sweden showed that *Staph. epidermidis* isolates recovered from cases of mastitis were genotypically identical to isolates recovered from the skin of milkers. This observation led them to suggest that *Staph. epidermidis* is a zoonotic organism. In contrast to the high prevalence of *Staph. epidermidis* in the human swab samples analyzed in this study, only one of the bovine isolates tested was identified as *Staph. epidermidis*. This may indicate that *Staph. epidermidis* is not a significant causative agent of IMI in our study area.

Overall, 15.2% of the humans sampled in this study were found to harbor *Staph. aureus*. It is reported that approximately 20% of healthy human individuals are persistent carriers of *Staph. aureus*, 30% are intermittent carriers, and 50% of individuals are never colonized by *Staph. aureus* (Kluytmans and Streulans, 2009; Graveland et al., 2011). Nasal carriage of *Staph. aureus* is recognized as a risk factor for acquiring infections and additionally plays a role in the dissemination of *Staph. aureus* in hospitals and the community (Zriouil et al., 2012).

The β -lactam antibiotics penicillin and ampicillin are used extensively for the prevention and treatment of IMI in dairy cows (IVS, 2015). In South Africa, more than half of the

intramammary preparations available for use comprise penicillin or ampicillin with or without dihydrostreptomycin (IVS, 2015). Furthermore, many of the preparations are available to farmers over the counter, making it difficult to monitor and control antimicrobial usage. In our study, resistance to penicillin was the most commonly detected resistance, with 28.8% of the *Staph. aureus* and 37.3% of CNS isolates showing phenotypic resistance to penicillin. This scenario parallels reports from other countries. Pyörälä and coworkers (2011) indicated that generally between one-third and two-thirds of bovine *Staph. aureus* isolates are resistant to penicillin, with lower percentages being reported in countries where strict antimicrobial policies have been implemented. Differences in the levels of bacterial resistance documented between studies are influenced by sampling strategies and bacterial populations but also differences in test methodology and the use of different interpretation criteria (Barkema et al., 2006). It is therefore suggested that comparisons between studies be restricted to studies that have used the same methodology and interpretation criteria (Schwarz et al., 2010).

It is of great significance, from both an animal and a public health perspective, that none of the staphylococcal isolates of bovine origin exhibited resistance to ceftiofur, the in vitro surrogate for examining the phenotypic susceptibility of isolates to methicillin (CLSI, 2014). Several studies have reported on the presence of methicillin-resistant staphylococci in milk samples, raising concerns over milk as a source of methicillin-resistant bacteria. In Switzerland, 47% of the CNS isolates evaluated in vitro, were found to be resistant to oxacillin (Frey et al., 2013).

Vanderhaeghen and co-workers (2010) found that almost 10% of herds sampled in Belgium to have cases of mastitis caused by MRSA. The in-herd prevalence of MRSA varied between 0 and 7.4%. Furthermore, all strains had characteristics typical of the zoonotic LA-MRSA strains belonging to clonal complex (CC) 398.

In South Africa, antimicrobial susceptibility data are sparse. Three studies have reported on the occurrence of MRSA from cases of bovine mastitis with prevalence rates varying between 1.1% and 93.2% (Petzer et al., 2007; Ateba et al., 2010; Schmidt, 2011). The high prevalence of 93.2% noted in one study is largely attributable to the sampling approach and the small sample size (Ateba et al., 2010). None of the studies confirmed the presence of the *mecA* gene in the isolates exhibiting phenotypic resistance. Generally, it is accepted that screening for the *mecA* gene is the most reliable method for the detection of methicillin resistance in staphylococcal isolates. Performing only phenotypic tests has previously led to the reporting of both false positive and false negative results (Vanderhaeghen et al., 2010).

Phenotypic resistance to antimicrobials other than penicillin and ampicillin was not common among the bovine staphylococcal isolates evaluated in our study. A low percentage of *Staph. aureus* (8.2%) and CNS (4.9%) isolates exhibited phenotypic resistance to streptomycin. Resistance to tetracycline was also noted, with 2.7% of the *Staph. aureus* and 8.8% of the CNS isolates showing phenotypic resistance to this antimicrobial. The observed resistance coincides with the availability of both of these antimicrobials in intramammary preparations in South Africa (IVS, 2015). None of the bovine *Staph. aureus* or CNS isolates exhibited resistance to cephalothin, clindamycin, erythromycin, linezolid or moxifloxacin, and only 2 isolates, 1 *Staph. hyicus* and 1 *Staph. xylosus*, were found to be phenotypically resistant to fusidic acid. The aforementioned antimicrobials have limited application in veterinary medicine but are used in human medicine to treat different types of staphylococcal infections (Lowy, 2013).

Multidrug resistance was more common among the CNS than the *Staph. aureus* isolates and more common among human isolates than bovine isolates. In total, 37 (39%) of the CNS isolates

of human origin were MDR, whereas only 2 *Staph. chromogenes* isolates of bovine origin were MDR. Two *Staph. aureus* isolates of bovine origin were MDR, whereas only a single *Staph. aureus* isolate from the human nasal swabs was found to be MDR. Interestingly, the human MDR *Staph. aureus* was isolated from a farm laborer on the same farm as the MDR bovine *Staph. aureus* isolate and displayed the same antimicrobial susceptibility profile. Future genotyping studies will be undertaken to determine the genetic relatedness of the bovine and human isolates and investigate possible bacterial transmission between hosts.

Antimicrobial susceptibility testing of isolates was supplemented by screening for specific antimicrobial resistance genes. None of the staphylococcal isolates evaluated were shown to carry the *vanA* and *vanB* genes, which confer vancomycin resistance to isolates. Considering the importance and reliance on vancomycin for the treatment of MRSA infections in human medicine, the lack of vancomycin resistance markers in both the bovine and human staphylococci analyzed in our study is therefore positive.

The correlation between the phenotypic susceptibility of isolates to penicillin with the presence of the *blaZ* gene was not absolute. A *Staph. sciuri* isolate of human origin and a *Staph. chromogenes* isolate of bovine origin were found to be phenotypically resistant to penicillin but negative for the presence of the *blaZ* gene. A possible explanation for this discrepancy could be a mutation of the primer-annealing site that prevented amplification. All staphylococcal test isolates were also screened for the presence of 2 tetracycline resistance determinants: *tetK* and *tetM*. The *tetK* gene was detected in the majority of the isolates showing phenotypic resistance to tetracycline, whereas *tetM* was not detected. Three isolates, two *Staph. chromogenes* and 1 *Staph. haemolyticus*, that were phenotypically resistant to tetracycline tested

negative for the presence of both *tetK* and *tetM*. This discrepancy could be due to the presence of other tetracycline resistance encoding genes, such as *tetL* or *tetO*, or due to a mutation in the primer-annealing site. The discrepancies observed between the phenotypic susceptibility of our test isolates and PCR screening for resistance genes highlight some of the shortcomings associated with the molecular detection of antimicrobial resistance. In many cases, antimicrobial resistance can be mediated by a number of different genes and mechanisms, some of which are still undescribed (Fluit, 2001).

One of the specific limitations associated with the determination of the antimicrobial susceptibility of veterinary bacterial isolates is the fact that there are currently very few antimicrobial breakpoints validated specifically for use with isolates of veterinary origin (Schwarz et al., 2010). At present, interpretive data are only available for the testing of mastitis pathogens against ceftiofur, penicillin-novobiocin and pirlimycin (CLSI, 2008). The latter antimicrobial is currently not registered for use in South Africa (IVS, 2015). Because of the lack of data, it is common practice to make use of breakpoints derived from human medicine to interpret in vitro data. This is not ideal and should be taken into consideration when interpreting results.

CONCLUSION

This study provides insight into the diversity of *Staphylococcus* spp. causing IMI in cows in South African dairy herds and communal animals. The predominance of *S. chromogenes* among the CNS isolates warrants further investigation to elucidate the epidemiology and virulence characteristics of strains circulating within herds. This study further demonstrated that

antimicrobial resistance amongst *Staphylococcus* spp. causing IMI was generally low. These results suggest the responsible usage of antimicrobials on dairy farms or the existence of management practices which do not favor the accumulation of resistance genes, or both. Because of the propensity of staphylococci to acquire antimicrobial resistance through the genetic exchange of DNA with material with compatible strains occupying the same microbiological niche, continued monitoring of bacterial populations is necessary.

ACKNOWLEDGEMENTS

The authors thank the University of Pretoria, RESCOM, the National Research Foundation (NRF) of South Africa, and the KZN Department of Agriculture & Rural Development for financially supporting this work. The MALDI-TOF MS work is based on the research supported in part by the NRF of South Africa (Grant specific unique reference number UID 74426).

Finally, the authors extend their thanks and appreciation to S. Monecke (Institute for Medical Microbiology and Hygiene, TU Dresden, Dresden, Germany) for his kind contribution of *Staph. aureus* control cultures.

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