# Distribution and Antibiotic Susceptibility Profiles of *Staphylococcus* spp Isolated from Unpasteurized Cow Milk Locally Consumed in Nkonkobe Local Municipality, South Africa

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## ABSTRACT

**Background**

Mastitis in cattle has been associated with infectious agents and trauma to secretory cells. Reduction in the quality and quantity of milk from dairy animals with concomitant adverse economic effects has been the leading implication of bovine mastitis. The leading microbes implicated in mastitis include *Staphylococcus* spp and coliforms among others. Consequently, this study aimed at the determination of the prevalence and in vitro susceptibility profiles of *Staphylococcus* spp in milk produced from dairy farms in the Nkonkobe region, Eastern Cape Province, South Africa.

**Methods**

A total of 384 milk samples were collected from three farms. Identification was performed based on conventional biochemical techniques employing the API Staph test kits whilst antibiotic susceptibility was determined by the disc diffusion assay.

**Results**

A total of 190 *Staphylococcus* spp were isolated in the study. *Staphilococcus aureus* (33.7%) was the most prevalent followed by S. xylosus (17.3%), while S hyicus (1.6%) and *S saprophyticus* (1.6%) were least isolated. Almost all isolates of *S aureus* 63 (98.4%) were resistant to penicillin G. How-

ever, 97% of the isolates were susceptible to chloramphenicol and streptomycin, while susceptibilities of 93.7% to both vancomycin and neomycin were observed. All the other species of *Staphylococcus* were generally susceptible to most of the antibiotics studied. Percentage susceptibility of 100% was demonstrated against vancomycin, amoxicillin, streptomycin, neomycin, and rifampin respectively. Multidrug resistance was a commonly observed phenomenon in the study with *S aureus* showing 98% prevalence.

**Conclusions**

These results indicate that *Staphylococcus* spp are prevalent in raw milk in the Nkonkobe region of the Eastern Cape Province, with isolates being resistant to several antibiotics which are used in the prevention and treatment of mastitis. These findings are of veterinary and clinical significance and therefore call for attention to address the situation.

## INTRODUCTION

Bovine mastitis is an inflammation of the mammary gland of cows caused by injury or infectious agents. The inflammatory response subsequent to infection causes an increase in somatic cell count (SCC) and damage to secretory cells adversely affecting milk production (Hortet and Seegers 1998; Seegers et al 2003). Several factors such as infectious agents, the environment, and management practices have been implicated in bovine mastitis (Bededa and Hiko 2011). Mastitis has been associated with the reduction in the quality and quantity of milk from cows, as well as impacting adversely the quality of milk products. Mastitis has been incriminated as an important disease constraint in dairy cow mainly due to microorganisms. Poultry, meat, and egg products as well as milk and milk products have been reported as common foods that may be contaminated with microorganisms (Capita et al 2002; Zakary et al 2011).

Microbes associated with mastitis and milk spoilage include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*, *Mycoplasma* species, *Streptococcus uberis* (Erskine 2002), coliforms (*Escherichia coli*, *Klebsiella* spp, *Enterobacter* spp, and *Serratia* spp), *Pseudomonas*, *Proteus* spp, and environmental *Streptococci* (Quinn et al 2002). Besides rendering milk and milk product unsuitable for human consumption, many of these organisms are responsible for diseases like streptococcal intoxication, colibacillosis, streptococcal sore throat, and brucellosis in humans. *Staphylococcus* spp is commonly found as skin and mucous membranes residents in cattle. However, despite their harmless nature as normal flora, pathogenic attributes ensue when epithelial or mucosal surface are exposed due to trauma. The production of a variety of virulence factors enabling adherence, colonisation, and invasion of the mammary cells of the bovine host by the *Staphylococcal* spp is required for disease progression.

Empiric antibiotic therapy is the preferred approach in the prevention and control of mastitis. However, complete control of mastitis-causing bacteria has been a daunting task as the manifestations of these pathogens are a common occurrence on farms. *Staphylococcus aureus* mastitis is the most prevalent, and antimicrobial resistance (AMR) has been one of the reasons for low cure rates (Barkema et al 2006). Antimicrobial resistance in bacteria is a public health hazard, and antimicrobial use is considered as a potentially important driver of AMR (Saini et al 2012). Being a highly significant challenge, antibiotic resistance decrease the effectiveness of drugs, and as such, increases morbidity and mortality associated with life threatening infections, thus compromising human (Collignon et al 2009) and animal health (Barkema et al 2006). Public hazards associated with the consumption of antibiotic resistant bacteria through contaminated milk includes allergic responses, changes in intestinal flora, and development of antibiotic resistant pathogens (Thirapatsakun 1999). Since the last decade, prevalence of antimicrobial resistance among foodborne pathogens has increased (Threlfall et al

2000; Yucel et al 2005; Nyenje et al 2012), possibly as a result of selective pressure created by the use of antimicrobials in animals (White et al 2002).

Foodborne diseases represent an important health problem, although the international impact of foodborne illness is difficult to estimate, as large numbers of illnesses remain underreported. Therefore, the true incidence of foodborne diseases is unknown (Gashaw et al 2008) due in part to the inability to distinguish between causative organisms following improper diagnosis, as common symptoms with other diseases may occur. The most common clinical presentation of foodborne diseases takes the form of gastrointestinal symptoms, which are generally mild and self-limiting. Hence antimicrobial therapy is not required (Nyenje et al 2012). However, therapy may be lifesaving in patients with underlying illness and those with prolonged febrile course of illness in whom invasive illness is suspected (Mølbak et al 2005). Hence, the objectives of this study were to determine the prevalence of *Staphylococcus* spp in cases of mastitis as well as their susceptibility to antimicrobial agents in a bid to guide empiric treatment in the Nkonkobe region, Eastern Cape, South Africa.

## MATERIALS AND METHODS

### Sampling Site and Sample Collection

Three commercial farms in the Amathole Local Municipality of the Eastern Cape Province (ECP) of South Africa were randomly selected for the studies. Cattle herd size and proximity to the University informed the choice for the sampling site. The ECP has been described as very rural, the second largest province in South Africa, with a high percentage of inhabitants living in poverty (ECSECC 2011).

Raw milk samples (50 mL) were collected in 100 ml sterile plastic container from 384 lactating cows from three private farms situated in the Eastern Cape Province, South Africa. The first farm had 700 dairy cows and produces about 22,000 L of milk per day, the second farm similarly had 700 dairy cows and produces about 18,000 L of milk per day, while the third farm had 100 dairy cows and produces about 2,000 L of milk per day. Only lactating cows were used in this study. The farms used in this study milked their cows twice daily (morning and noon) for small scale public sells and large scale sells to dairy industries. Samples were collected in accordance with the in-house procedures of each farm using rotary milking parlours with 40-bail units. The research activity was performed in accordance with the ethical guidelines outlined by the National Committee for Research Ethics in Science and Technology of South Africa, and in furtherance, all experiments adhered to the University of Fort Hare's guidelines on health and safety. Samples were kept at 4°C and transported immediately to the University of Fort Hare (UFH) microbiology laboratory in a cooler box with ice packs. Upon arrival to the Laboratory, samples were analysed immediately.

### Isolation and Identification of Staphylococci species

A total of 384 milk samples were collected from lactating cows and analysed in the laboratory. Ten microliters (10 µL) of each milk sample was cultured on Mannitol Salt agar (Merck, Germany), and blood agar plates supplemented with 5% sheep blood (Merck, Germany). All plates were incubated at 37°C for 24 to 48 h. Gram stain, culture characteristics, and coagulase test using fresh rabbit plasma (tube method) were used for the presumptive identification of all isolates (National Mastitis Council 1999). API Staph (Biomerieux Inc., Quebec) was used to confirm the isolates. The tests were performed as per manufacturer's instruction and data interpretation was performed using the Analytical profile index (API) database (V4.1) with the apiwebTM identification software.

### Antibiotic Susceptibility Testing

Sensitivity testing using the disk diffusion method was done according to the method described by Ndip et al (2008) in accordance with the CLSI (2006). Briefly, a small inocu-

lum of each bacterial isolate was emulsified in 3mL sterile normal saline in Bijou bottles and the density compared to a barium chloride standard (0.5 McFarland). A sterile cotton swab was dipped into the standardized solution of bacterial cultures, and used to evenly inoculate Mueller-Hinton plates (Biotec, England), and allowed to dry. Thereafter, antibiotic disks with the following drug contents: eg, vancomycin (30 µg); kanamycin (30 µg); ampicillin (10 µg); amoxicillin (20 µg); gentamycin (10 µg); tetracycline (30 µg); chloramphenicol (30 µg); streptomycin (10 µg); neomycin (30 µg); rifampin (5 µg); erythromycin (15 µg); and penicillin G (10 IU) (Oxoid, England) were placed on the plates, spacing them well to prevent the overlapping of inhibition zones. The plates were incubated at 37 °C for 24 hours and the diameters of inhibition zones were recorded.

## RESULTS

### Prevalence of *Staphylococcus* species

Of the 384 raw milk samples tested, 190 (49.5%) were positive for *Staphylococcus* spp. The isolates were identified as *S aureus* 64 (33.7%), *S xylosus* 33 (17.3%), *S chromogenes* 27 (14.2%), *S hominis* 24 (12.6%), *S warneri* 24 (12.6%), S sciuri 6 (3.2%), S epidermidis 6 (3.2%), S hyicus 3 (1.6%), and *S saprophyticus* 3 (1.6%).

### Antimicrobial Profiles of *Staphylococcus* species

Almost all isolates of the *S aureus* 63 (98.4%) were resistant to penicillin G. Of the 64 isolates, 97% were susceptible to chloramphenicol and streptomycin, while susceptibilities of 93.7% to both vancomycin and neomycin

***Table 1.*** *Antibiotic profiles of Staphylococcus spp. isolated from raw milk from cows in the Eastern Cape Province, South Africa.*

| Antibiotic | Number (%) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus* n=64 | | | *S. xylosus* n= 33 | | | *S. hominis* n= 24 | | | *S. warneri* n= 24 | | | *S. chromogenes* n= 27 | | |
| | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R |
| Vancomycin | 60 (93.7) | 1 (1.7) | 3 (4.6) | 33 (100) | 0(0) | 0(0) | 24(100) | 0(0) | 0(0) | 24 (100) | 0(0) | 0(0) | 27 (100) | 0(0) | 0(0) |
| Kanamycin | 52 (81.2) | 8(12.5) | 4(6.3) | 33 (100) | 0(0) | 0(0) | 24 (100) | 0(0) | 0(0) | 24 (100) | 0(0) | 0(0) | 27 (100) | 0(0) | 0(0) |
| Ampicillin | 32(50) | 0(0) | 32(50) | 0(0) | 32(97) | 1 (3) | 0(0) | 23(95.8) | 1 (4.2) | 24(100) | 0(0) | 0 (0) | 27(100) | 0(0) | 0 (0) |
| Amoxillin | 45(70.3) | 1(1.6) | 18(28.1) | 33 (100) | 0(0) | 0(0) | 24 (100) | 0(0) | 0(0) | 24 (100) | 0(0) | 0(0) | 27 (100) | 0(0) | 0(0) |
| Gentamycin | 59(92.2) | 0(0) | 5(7.8) | 22 (69.7) | 10 (30.3) | 1 (3) | 12 (50) | 10 (41.7) | 2 (8.3) | 24 (100) | 0(0) | 0(0) | 27 (100) | 0(0) | 0(0) |
| Tetracycline | 49(76.6) | 0(0) | 15(23.4) | 32 (97) | 0 (0) | 1 (3) | 23 (95.8) | 0 (0) | 1 (4.2) | 24 (100) | 0(0) | 0(0) | 27 (100) | 0 (0) | 0(0) |
| chloramphenicol | 62(97) | 0(0) | 2(3) | 16 (48.5) | 17 (51.5) | 0(0) | 16 (66.7) | 8 (33.3) | 0(0) | 24 (100) | 0(0) | 0(0) | 27 (100) | 0(0) | 0(0) |
| Streptomycin | 62(97) | 2(3) | 0(0) | 33 (100) | 0(0) | 0(0) | 24 (100) | 0(0) | 0(0) | 24(100) | 0(0) | 0(0) | 27(100) | 0(0) | 0(0) |
| Neomycin | 60(93.7) | 0(0) | 4(6.3) | 33(100) | 0(0) | 0(0) | 24 (100) | 0(0) | 0(0) | 24(100) | 0(0) | 0(0) | 27(100) | 0(0) | 0(0) |
| Erythromycin | 23 (36) | 9(14) | 32(50) | 31 (94) | 0(0) | 2(6) | 22 (91.7) | 0(0) | 2(8.3) | 22 (91.7) | 0(0) | 2(8.3) | 22 (81.4) | 3(11) | 2(7.4) |
| Rifampin | 15(23.4) | 0(0) | 49(76.6) | 33 (100) | 0(0) | 0(0) | 24 (100) | 0(0) | 0(0) | 24 (100) | 0(0) | 0(0) | 27 (100) | 0(0) | 0(0) |
| Penicillin G | 1(1.6) | 0(0) | 34(53) | 17 (51.5 ) | 14(42.4) | 2 (6) | 14 (58.3 ) | 8 (33.3) | 2 (8.3) | 18 (75 ) | 0(0) | 6 (25) | 18 (75 ) | 3 (11) | 6 (22.2) |

***Table 2.*** *Antibiotypes of Staphylococcus species isolated from cow milk in the Eastern Cape Province, South Africa.*

| No | Antibiotype | Number (%) | | | | |
|---|---|---|---|---|---|---|
| | | *S. aureus* | *S. xylosus* | *S. hominis* | *S. warneri* | *S. chromogenes* |
| A1 | $PG^R E^R$ | 32(50) | 0(0) | 2(8.3) | 2(8.3) | 2(7.4) |
| A2 | $PG^R VA^R E^R$ | 2(3.1) | 2(6) | 0(0) | 0(0) | 0(0) |
| A3 | $A^R PG^R VA^R E^R$ | 2 (3.1) | 0(0) | 0(0) | 0(0) | 0(0) |
| A4 | $K^R PG^R VA^R E^R$ | 2 (3.1) | 0(0) | 0(0) | 0(0) | 0 |
| A5 | $PG^R VA^R E^R AP^R$ | 0(0) | 1(3) | 0(0) | 0(0) | 0(0) |
| A6 | $PG^R VA^R E^R T^R$ | 3(5) | 0(0) | 0(0) | 0(0) | 0(0) |
| A7 | $AP^R G^R T^R E^R PG^R$ | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| A8 | $K^R PG^R VA^R E^R AP^R$ | 4(6.3) | 0(0) | 0(0) | 0(0) | 0(0) |
| A9 | $K^R PG^R VA^R E^R T^R$ | 2(3.1) | 0(0) | 0(0) | 0(0) | 0(0) |
| A10 | $PG^R Am^R E^R T^R AP^R$ | 5(8) | 0(0) | 0(0) | 0(0) | 0(0) |
| A11 | $PG^R Am^R E^R NE^R T^R$ | 4(6.3) | 0(0) | 0(0) | 0(0) | 0(0) |
| A12 | $PG^R Am^R E^R T^R AP^R$ | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| A13 | $K^R PG^R Am^R E^R NE^R AP^R$ | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| A14 | $K^R PG^R Am^R E^R T^R AP^R$ | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| A15 | $K^R PG^R Am^R E^R NE^R T^R$ | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| A16 | $PG^R Am^R E^R T^R AP^R C^R$ | 3(5) | 0(0) | 0(0) | 0(0) | 0(0) |
| A17 | $K^R PG^R Am^R E^R NE^R T^R AP^R$ | 5(8) | 0(0) | 0(0) | 0(0) | 0(0) |
| A18 | $K^R PG^R Am^R E^R NE^R T^R AP^R GM^R$ | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |

*Am, amoxicillin; C, chloramphenicol; K, kanamycin; PG, penicillin G; VA, vancomycin; E, erythromycin; NE, neomycin; T, tetracycline; AP, ampicillin; GM, gentamicin.*

were observed, with the rest of the isolates displaying various degrees of susceptibility or resistance (Table 1). It was interesting to note that all the other species of *Staphylococcus* were generally susceptible to most of the antibiotics studied. Percentage susceptibility of 100% was demonstrated against vancomycin, amoxicillin, streptomycin, neomycin, and rifampin respectively. Moderate to low resistances of 25%, 22.2%, 8.3%, and 6% were noted for *S warneri*, S. chromogenes, S. Hominis, and S. xylosus respectively against penicillin G. Various degrees of susceptibility or resistance were noted for the other species as well (Table 1).

Drug resistance was a common phenomenon observed in 63 (98%) *S aureus* 3 (9.09%) *S xylosus*, 3 (12.5%), 2(8.3%) *S Warneri*, and 2 (7.47%) *S chromogenes* isolates. Eighteen antibiotic resistance patterns were obtained (Table 2). Biotype A1: PGR ER (penicillin G and erythromycin) was the most predominant in *S aureus* 32 (50%), *S xylosus* 2 (6%), *S hominis* 2 (8.3%), *S Warneri* 2 (8.3%), and *S chromogenes* 2 (7.4%). The least resistant patterns for *S xylosus* 1(3%) were observed in biotype A5: PGR VAR ER APR (penicillin G, vancomycin, erythromycin, and ampicillin), while for *S aureus* 2 (3.1%) were demonstrated by A2 (PGR VAR ER), A3 (AR PGR VAR ER), A4 (KR PGR VAR ER), and A9 (KR PGR VAR ER TR) respectively demonstrated the same pattern.

## DISCUSSION

Mastitis is the most prevalent disease of dairy cows, and remains one of the most

common reasons to withdraw cows from production (Pol and Ruegg 2007). Clinical mastitis is characterized by abnormalities in milk, while subclinical mastitis is characterized by normal appearance of milk with increased numbers of somatic cells. Bovine mastitis caused by Staphylococci from a milker's hands (Lee et al 2012) can result in both clinical and subclinical disease (Wilson et al 1997), and also pose serious public health concern. In an increasingly competitive global market, it is important to produce safe food with economic importance. Food surveillance for microbial contamination is important for public health protection and consumer interest (Addis et al 2011).

*Staphilococcus aureus* is a contagious mastitis agent that represents a risk for mastitis outbreaks occurring in a herd and frequently causing intramammary infections (Smith et al 2005). *Staphilococcus aureus* was detected in 33.7% of the samples investigated in our study. However, this value is lower than those detected in Ontario, Canada (92%) (Kelton et al 1998), and Prince Edward Island, Canada (70%) (Keefe et al 1998), but higher than in a study done in Sweden (18.5%) (Persson Waller et al 2011). This differences could be linked to the varied veterinary and hygienic measures implemented in different countries (Chatterjee and Otto 2013).

*Staphilococcus aureus* may also cause human infections, and is associated with community acquired, livestock acquired, and nosocomial morbidity and mortality (Zadoks et al 2000; Chatterjee and Otto 2013). A range of illnesses in humans from minor skin infections, such as pimples, impetigo, boils, cellulites, furuncles, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), and septicaemia have been associated with *S aureus* (Chamber 2001).

Several authors have reported on the isolation of other members of staphylococci, which at times are generally reported as coagulase negative Staphylococci (CNS) (Ebrahimi et al 2007). However, CNS includes several *Staphylococcus* spp, but is not limited to *S hominis*, *S xylosus*, *S warneri*, *S chromogenes*, and *S saprophyticus*. The role of CNS in mastitis has greatly increased during the last years with this group of bacteria reported to cause subclinical mastitis (Smith, 2001). However, some authors reported a high prevalence of clinical cases caused by CNS (Bradley and Green 2001; Malinowski et al 2001). In our study, 17.3% of *S xylosus* were isolated from raw milk investigated. This value is higher than the value (0.8%) reported by Menzies et al. (2001). *S xylosus* has been implicated in urinary tract infections in women (Rupp et al 1992), sheep dermatitis, and bovine mastitis (Gourreau et al 1994).

*S chromogenes* has been isolated from the udders of unbred, pregnant, or freshly calved heifers (Trinidad et al 1990). *S chromogenes* was found to be the most prevalent pathogen associated with CNS intramammary infection (IMI) in Washington State (Quirk et al 2012). In our study, *S chromogenes* was the third most prevalent (14.2%) CNS isolated from milk. Supré et al (2012) reported that *S chromogenes* caused more persistent IMI than other CNS. This may be due to the fact that *S chromogenes* is more resistant to 1% iodophore, a chemical used for post-milking teat disinfection (Quirk et al 2012).

*S hominis*, which accounted for 12.6% of the isolates in our study, occurs very commonly as a harmless commensal on human and animal skin. However, like many other CNS, *S hominis* may occasionally cause infection in patients whose immune system is compromised by chemotherapy or predisposing illness. Kenar et al (2012) reported a 7.4% isolation of *S hominis* from lactating cows in Turkey. *S warneri* was reported as a potential cause of bovine abortion (Barigye et al 2007). Several reports have described the organism as one of the most common CNS associated with blood infections in humans (Hall et al 1987; Martin et al 1989). *S*

*warneri* was isolated from bovine bulk tank milk in Norway (Bjorland et al 2005). In our study, 12.6% of *S warneri* was isolated.

Isolation of bacterial agents and antimicrobial susceptibility test are important in reducing the occurrence of drug resistance and increase the production of milk and milk products. Failure to do so may cause the emergence of multidrug resistance in these organisms. Antibiotic treatment of bovine mastitis contributes substantially to the overall antibacterial drug use in veterinary medicine in many countries. It is generally accepted that selection pressure from the use of antibiotics is a main factor in the development of antibiotic resistance. The remarkable ability of Staphylococci to acquire antibiotic resistance limits therapeutic options (Anderson-Berry 2011; Jain et al 2011) and mastitis caused by *staphylococcal* infections has increased the financial burden on economic systems worldwide.

The distribution of antimicrobial resistant staphylococci presents a challenge to both human and animal health professionals. A high resistance of *S aureus* to penicillin G (98.4%) was observed in our study. The resistance of staphylococci to β-lactam antibiotics (penicillin G and ampicillin) may be attributed to the production of β-lactamase, an enzyme that inactivates penicillin and closely related antibiotics. Moreover, this could be associated with the predominant use of penicillin for treatment of animal diseases. This result agrees with others which equally noted an increase in resistance to β-lactam antibiotics (Alekshun and Levy 2000; Jovetic et al 2010; Al-Thani, and Al-Ali 2012).

Antibiotic resistance to rifampin (76.6%) was the second highest resistance encountered in our study. Rifampin acts by inhibiting RNA synthesis by binding to a subunit of the bacterial RNA polymerase. Ampicillin and erythromycin inhibited 50% of *S aureus*. Erythromycin is a macrolide containing large cyclic molecules. The resistance of organisms to macrolides is mediated by chloramphenicol acetyl transferase enzyme in some organisms. However in other Gram positive cocci, resistance is primarily due to either plasmid-encoded mef and erm genes for efflux or alteration in the 23S rRNA target by methylation of the two adenine nucleotides in the RNA (Scalet et al 2010).

Multidrug resistance was a common phenomenon observed in 63 (98%) *S aureus* 3 (9.09%) *S xylosus*, 3 (12.5%), 2(8.3%) *S Warneri*, and 2 (7.47%) *S chromogenes* isolates. Eighteen antibiotic resistance patterns were obtained (Table 2). Biotype A1: PGR ER (penicillin G and erythromycin) was the most predominant in *S aureus* 32 (50%), *S xylosus* 2 (6%), *S hominis* 2 (8.3%), *S warneri* 2 (8.3%), and *S chromogenes* 2 (7.4%). The least resistant patterns for *S xylosus* 1(3%) were observed in biotype A5: PGR VAR ER APR (penicillin G, vancomycin, erythromycin, and ampicillin), while for *S aureus* 2 (3.1%) were demonstrated by A2 (PGR VAR ER), A3 (AR PGR VAR ER), A4 (KR PGR VAR ER), and A9 (KR PGR VAR ER TR) respectively demonstrated the same pattern.

The results indicated alarming drug-resistance frequencies to at least two or more of the test antibiotics. This is of major concern to the public, as the milk may act as reservoir of resistant strains that can be transmitted to humans upon ingestion of the contaminated milk. They also suggest that incidence of antibiotic resistance in *staphylococcus* spp is relatively high. It is, however, of great concern that the range of antibiotics to which resistance has been acquired is wide and expanding, including a number of antibiotics used to treat mastitis in the Eastern Cape (penicillin, kanamycin, ampicillin, and amoxicillin). This may have future implications for the effective treatment of mastitis if these resistant strains persist in milk and if transferred to humans, several diseases would be difficult to control.

## CONCLUSION

*Staphylococcus* spp is prevalent in raw milk in the study area. CNS species are resistant at high rates to the beta-lactam antibiot-

ics, which are used in the prevention and treatment of mastitis. These findings are of veterinary and clinical significance and, therefore, call for attention to address the situation.

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## CONFLICT OF INTERESTS

We declare that there are no conflicts of interests.

## AUTHOR'S CONTRIBUTIONS

AP: Carried out the sample collections, molecular assays and drafting of the first manuscript draft. UUN: Interpreted the results and prepared the final version of the manuscript. AIO: Co-designed the work and proof read the final version of the manuscript. EG: Designed the research, supervised the execution of the research and proof read the final version of the manuscript.

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