Antimicrobial resistance profiles of staphylococci from subclinical mastitis in commercial dairy farms in Goromonzi District, Zimbabwe

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SUMMARY

A cross-sectional study aimed at isolating and estimating the prevalence of multidrug resistant coagulase negative staphylococcus (CNS) species from subclinical mastitic dairy cows at six selected commercial farms in Goromonzi District was conducted from January to March 2024. Three hundred milk samples from cows with subclinical mastitis, classified based on the somatic cell counts, were collected and analyzed. The CNS bacteria were identified using colony morphology, gram staining and biochemical tests. The antibiotic ssusceptibility profiles of the identified bacteria were tested against 15 antibiotics using the Kirby-Bauer disc diffusion method and were interpreted using the CLSI guidelines. Six CNS species were identified as: *Staphylococcus epidermidis, S. haemolyticus, S. simulans, S. xylosus*, *S. saprophyticus* and *S. chromogenes.* The overall prevalence of CNS species across all six farms was 20.3% (61/300; 95%CI: 15.8 – 24.8%). Amongst all the CNS species (n=61), *S. epidermidis* (55.7%) was the most isolated, followed by *S. xylosus* (11.9%), *S. chromogenes* and *S. saprophyticus* with 9.8% each, then *S. simulans* (8.2%) and the least isolated was *S. haemolyticus* with 3.3 % . The overall prevalence of multidrug resistance (resistant to >2 antimicrobials) of CNS species was 11.5% (7/61; 95%CI: 4.6 – 18.4%) signaling the propensity of resistant genes to spread to the major mastitis pathogens. Thus, proper antibiotic stewardship is required to prevent escalation of AMR in the dairy sector.

Keywords: antimicrobial resistance, mastitis, dairy cattle, coagulase negatative staphylococcus, Zimbabwe

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INTRODUCTION

Mastitis is one of the most common and economically important diseases of dairy cattle globally (Chen and Han, 2020; Dego and Vidlund 2024). Dairy operation profitability is lowered by lower milk output, increased treatment expenses, and forced culling, among other factors that are frequently connected to animal health problems like mastitis or reproductive issues (Cobirka et al., 2020; Fasseha et al., 2021; Morales-Ubaldo et al., 2023). The disease can present mainly as clinical mastitis (CM) or subclinical mastitis (SCM) (Girma and Tamir, 2022). Clinical mastitis results in observable abnormality in both the milk and the udder. The signs observed are red swollen and hot udder, pain during milking which can cause cows to stump their feet, color change in milk, presence of clots and pus in milk and can be bloody or watery (Girma and Tamir 2022; Chen et al., 2023). The prevalence of subclinical mastitis varies by study and region. Previous studies done reported SCM prevalences in dairy herds in Zimbabwe (16.3%) (Katsande et al, 2013), South Africa (17.8%) (Ramuada et al, 2025) and up to 50% in some Chinese herds (Chen and Han, 2020). This non-symptomatic inflammation of the udder is associated with a high somatic cell count (SCC), imposing milk quality penalties and resulting in economic losses to the farmer (Fasseha et al., 2021; Girma and Tamir 2022).

The etiology of bovine mastitis is multifactorial but bacterial pathogens account for more than 90% of all mastitis diagnosis (Chen and Han, 2020; Vidlund et al, 2022; Dego and Vidlund 2024). Most common bacteria that cause bovine mastitis were historically classified as environmental and contagious pathogens where the latter are part of the normal flora of cows that are capable to colonize the mammary gland and spread through direct contact from an infected cow to another, contaminated milking machines or the hands of milkers (Cobirka et al., 2020). Environmental pathogens of mastitis can be spread from a contaminated environment (Cobirka et al., 2020). Common bacterial agents causing mastitis globally include *Escherichia coli, Klebsiella* sp., *Staphylococcus aureus,* coagulase negative staphylococcus (CNS), *Corynebacterium bovis, Streptococcus agalactiae, Streptococcus uberis*, and other streptococci species (Fasseha et al., 2021; Chen et al., 2023).

Coagulase negative staphylococcus species constitute some of the normal commensals of the skin of the teat and external orifice of the teat canal (De Buck et al., 2021). CNS are neither categorized as the major pathogens of mastitis but rather as opportunistic bacteria which lack many of the virulence factors associated with *S. aureus* and are rarely associated with severe infections, although they can elicit an inflammatory response and result in an elevated SCC in milk (De Buck et al, 2021). CNS possesses both traits of environmental and contagious pathogens. CNS refers to a group of bacterial species that may behave differently in the udder. Recent research has shown that only a few species usually account for intramammary infections (IMI). Frequently isolated species in IMI include. *S. chromogenes, S. simulans, S. epidermidis, S. haemolyticus* and *S. xylosus* (Hamel et al., 2020; Dego and Vidlund, 2024).

An increase in the importance of CNS as mastitogenic bacteria has been reported throughout the world (Bhavana and Chaitanya, 2022; Idamokoro, 2022; Dego and Vidlund, 2024). CNS species are often referred to as minor mastitogenic pathogens, however recent literature has frequently identified CNS isolates in milk from multiparous cows and primiparous heifers with elevated somatic cell counts and subclinical mastitis (Dego and Vidlund, 2024). To date, antimicrobial therapy has been an effective strategy for controlling intramammary infections (Chen and Han, 2020) however, there is an increase in antimicrobial resistance to a number of antibiotics that are commonly used in Zimbabwe and worldwide (Gufe et al., 2020; Ounah, 2024). Previous studies reported that *S. aureus* isolated from dairy cows with SCM and CM were less resistant than CNS against commonly used antimicrobials (Taponen *et al*., 2007). Furthermore, some researchers have found that CNS can harbor genes that can confer antimicrobial resistance to major mastitogenic pathogens including *S. aureus*, potentially acting as a genetic reservoir for MDR (Fisarova *et al*., 2019). Therefore, the objective of this study was to investigate the antimicrobial resistance profiles of CNS species isolated from milk samples of cows with subclinical mastitis from selected commercial farms in the Goromonzi District, Zimbabwe.

MATERIALS AND METHODS

Study design and study area

A cross-sectional study aimed at determining the prevalence of multidrug resistance in coagulase negative staphylococci (CNS) bacteria isolated from subclinical mastitic dairy cows from selected commercial dairy farms was conducted in January to March 2024. The farms are in a rural community from Goromonzi District in Mashonaland East Province, Zimbabwe. Goromonzi district lies between coordinates, latitude and longitude, -17.856242, 31.3798921 and is situated in the agro-ecological region II which receives between 700 and 1000mm of rainfall annually.

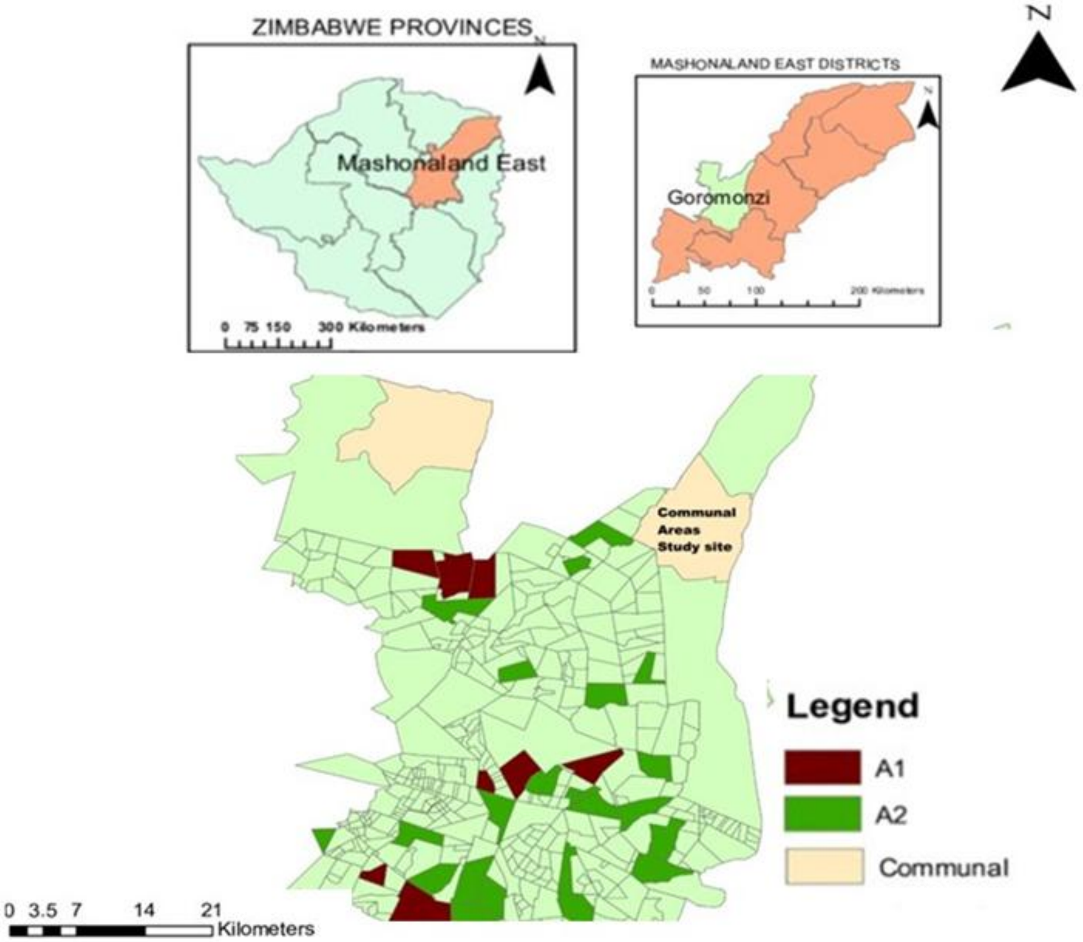

Figure 1: Map showing Zimbabwe Provinces and Goromonzi District

The district contains three farm systems: communal, A1, and A2 farms (Figure 1). In a communal farming system, less dairy production is conducted, with the majority of farming being for subsistence. This also applies to the A1 farm system. However, A2 farms are where the majority of commercial dairy farming takes place, and there are an estimated total of 10,000 dairy animals on commercial dairy farms.

Study population

The study population were dairy cows with subclinical mastitis and this was based on the somatic cell count done by the Dairy Service Section. Six commercial dairy farms were purposively sampled based on the results of the somatic cell counts. The basis of the selection of farms sampled was on farms that are producing and supplying milk to dairy production centers as the research commenced as well as accessibility of the farms as well as farms with high somatic cell counts from monthly Dairy Services inspections. The somatic cells were determined by the Ekomilk scan somatic cells analyzer and cows with somatic cell counts above 250 000 cells per ml were sampled.

Sample size determination

Prior to sample collection, the sample size of 300 was calculated using the estimated previous prevalence of 0.27 (Katsande *et al*., 2013). Formula for sample size calculation: $N = Z^2 (p(1-p))/ e^2$,
where N is the total number of cows to be sampled, the standard normal variate (Z) =1.96

at a 95% confidence level, the expected proportion of multi-drug resistant CNS in the population (p) =0.27 and the desired precision (e) =0.05. From this calculation, a total of 303 samples was supposed to be sampled however, the sample size was rounded off to 300 samples.

Milk sampling

Cows with subclinical mastitis were purposively selected and composite milk samples for bacterial culture were aseptically collected only from 300 cows with subclinical mastitis in six commercial dairy farms. Teats were initially washed using clean running water and wiped with sterile disposable paper towels (Kudinha and Simango, 2002). Teats were disinfected using 70% ethyl alcohol prior to milking. The first three streams of milk were discarded in order to minimize bacterial contamination from the skin around the teat and teat canal (Katsande *et al*, 2013). Composite milk samples were then collected aseptically and kept in sterile universal bottles. The samples were transported in cooler boxes on ice packs (approximately 4℃) to the Central Veterinary Laboratory in Harare where all the bacteriological laboratory tests were performed. Milk samples were promptly stored in the refrigerator at 4 ℃ overnight and analyzed within 12 to 16 hours from collection.

Bacteriological culture, isolation and identification

The refrigerated samples were acclimatized to room temperature (25-26^{0}C) for 30 minutes. The milk was mixed by gentle inversions of the containers. About 10 µL of each composite milk sample was streaked on 5% Sheep-Blood Agar (CM0854 Oxoid) and MacConkey Agar (CM0317 Oxoid) for mastitogenic bacterial culture, using the quadrant streaking method for all the samples (Bhavana and Chaitanya, 2020). The agar plates were incubated for 24 hours at 37 ˚C and were examined for bacterial growth and morphological features (colony size, color, shape and haemolytic characteristics). A sample was considered to be positive for mastitogenic pathogen(s) if there was a growth of a single colony of the potential pathogen which was confirmed using biochemical tests (Katsande *et al.*, 2013). Growth of more than one type of colony was considered as mixed growth and was not analyzed for further investigations. Respective colonies were selected, sub-cultured on Blood Agar and incubated at 37 ˚C for 24 hours in order to obtain pure cultures. The gram stain test was undertaken on the resultant colonies and the bacteria were distinguished based on the gram stain reaction, cellular morphology and the bacterial arrangement. The gram-positive bacteria were sub-cultured on Mannitol Salt Agar (CM0085 Oxoid). Catalase, coagulase test and other biochemical tests were carried out to identify the CNS species as described by (Barrow and Feltham 1993). The coagulase test was used to distinguish between coagulase positive and coagulase negative bacteria. The *S. aureus* ATCC 25923 strain was used as a control during the study.

Antimicrobial susceptibility testing (Disc diffusion assay)

The Kirby-Bauer agar disc diffusion method (*in-vitro* antibiotic sensitivity test) was carried out in order to determine the susceptibility of the bacteria to the most commonly used and available antibiotics in veterinary practices in Zimbabwe. A loopful of the bacterial isolates was transferred to peptone water in tubes and incubated at 37℃ for 2 hours. The peptone water with the bacterial suspension was then plated onto Mueller Hinton Agar (CM0337 Oxoid) using sterile cotton swabs with turbidity adjusted to 1.5 x 10^8 CFU/mL, compared to 0.5 McFarland standards. Antibiotic-impregnated discs (Oxoid) were applied onto the plates using an antibiotic injector. The plates were incubated for 24 hours at 37℃. The antibiotics used were Kanamycin (K30), Gentamicin (GM10), Trimethoprim Sulpha (SXT25), Cotrimoxazole (TS25), Oxacillin (OX1), Cloxacillin (OB5), Ampicillin (AMP25), Penicillin G (PG10), Meropenem (MER10), Ertapenem (ETP30), Cefotaxime (CTX30), Chloramphenicol (C30), Ciprofloxacin (CIP5), Vancomycin (VAN10) and Erythromycin (ERY15). The zones of growth inhibition were measured using a vernier caliper, as the diameters (in millimeters) of the zones surrounding the antibiotic discs where growth was absent. The results were interpreted as resistant, intermediate and sensitive to the different antibiotics that were used using the Clinical and Laboratory Standards Institute (CLSI) breakpoints of 2023. CNS isolates with intermediate breakpoints may become resistant overtime since there have been reports of weak regulations on antimicrobial usage hence, the intermediate CNS isolated were regarded as resistant (Gizaw *et al*., 2020).

Data analysis

Data on CNS isolates and antimicrobial susceptibility tests were recorded on Microsoft Excel spreadsheet where edits and descriptive statistics were done.

RESULTS

Isolation of CNS species in subclinical mastitis across the six farms.

The total number of CNS species isolated from all six farms was 20.3% (61/300; 95%CI: 15.8 – 24.8%) all mastitis milk samples investigated. The CNS species identified were *S. haemolyticus*, *S. epidermidis*, *S. simulans*, *S. xylosus*, *S. saprophyticus* and *S. chromogenes* as shown on figure 1. *Staphylococcus epidermidis* was the most isolated species across all farms, accounting for 34 out of the 61 isolates.

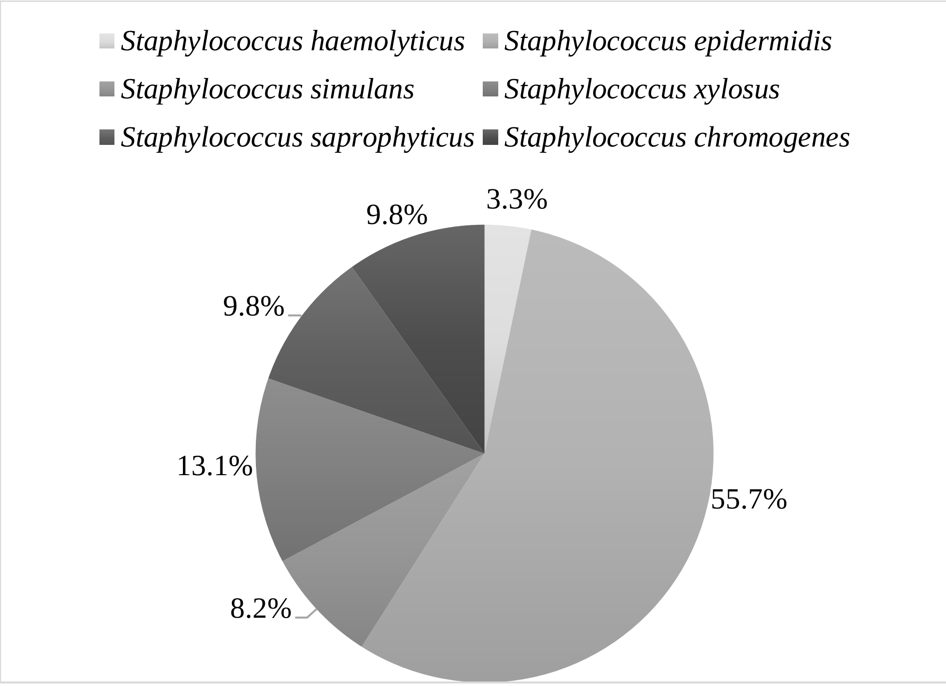

Figure 1. Diversity of CNS species isolated from mastitis milk

Distribution of the different CNS **species by farms**

Farm A had the highest number of CNS species isolates 34.3% (21/61; 95%CI: 22.6 – 46.2%), with *Staphylococcus epidermidis* (12 isolates) being the most isolated species. Farms B and C each had a total of four isolates whilst Farm D had seven isolates. Lastly Farms E and F had 10 and 15 isolates respectively largely dominated by *Staphylococcus epidermidis* (shown in figure 2).

Prevalence of individual species across the six farms.

The overall prevalence of CNS species across all the farms was 20.3% (61/300; 95%CI: 15.8 – 24.8%). Table 1 shows the individual CNS prevalences across all the six farms. *Staphylococcus epidermidis* was the most prevalent species, making 55.7% of the total CNS isolates across all farms. *Staphylococcus haemolyticus* was the least prevalent, making up only 3.3% of the isolates. Farm A had the

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highest overall prevalence of 34.4% of CNS species, with *Staphylococcus epidermidis* (57.1%) being the most isolated species followed by Farms F and Farm E which had overall prevalence of 24.6% and 16.4% respectively. In both Farms E and F, *S. epidermidis* had the highest prevalence of 60%. Lastly Farms B, C and D had the least overall prevalence with *S. epidermidis* contributing the larger percentage.

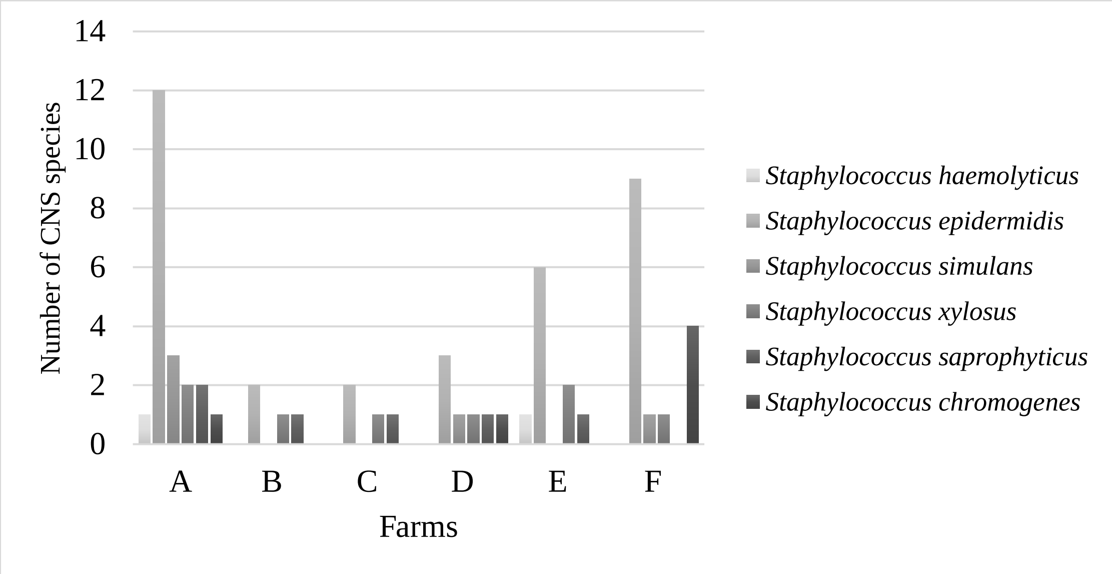

Figure 2. **Distribution of CNS species isolated from mastitis milk by farms**

Table 1. Prevalence of individual CNS species isolated from mastitis milk across the six farms in Zimbabwe

	Number of isolates (%)						
	Farms						
Pathogens	**A**	**B**	**C**	**D**	**E**	**F**	**Total**
S. haemolyticus	1(4.8)	0	0	0	1 (10.0)	0	2(3.3)
S. epidermidis	12(57.1)	2(50.0)	2(50.0)	3(42.9)	6(60.0)	9(60.0)	34(55.7)
S. simulans	3(14.3)	0	0	1(14.3)	0	1(6.7)	5(8.2)
S. xylosus	2(9.5)	1(25.0)	1(25.0)	1(14.3)	2(20.0)	1(6.)	8(13.1)
S.saprophyticus	2(9.5)	1(25.0)	1(25.0)	1(14.3)	1(10.0)	0	6(9.8)
S.chromogenes	1(4.8)	0	0	1(14.3)	0	4(26.7)	6(9.8)
Total Farm CNS prev	**21(34.4)**	**4(6.6)**	**4(6.6)**	**7(11.5)**	**10(16.4)**	**15(24.6)**	**61(100)**

Table 2. Antimicrobial resistance profiles of CNS species isolated from mastitis milk in Zimbabwe

Antimicrobial drugs	Number of resistant isolates (%)						
	S. epidermidis (n=34)	*S. haemolyticus* (n=2)	*S. simultans* (n=5)	*S. xylosus* (n=8)	*S. saprophyticus* (n= 6)	*S. chromogenes* (n=6)	Total
K30	8(23.5)	1(50)	0	3(37.5)	3(50)	3(50)	18(29.5)
TS25	6(17.7)	0	0	4(50)	1(16.7)	1(16.7)	12(19.7)
C30	2(5.9)	0	0	0	0	1(16.7)	3(4.9)
OB5	22(64.7)	0	5(100)	4(50)	3(50)	6(100)	40(65.6)
ETP30	0	0	0	0	0	0	0
CTX30	2(5.9)	2(100)	0	4(50)	0	0	8(13.1)
AMP25	14(41.2)	2(100)	2(40)	3(37.5)	2(33.3)	3(50)	26(42.6)
ERY15	6(17.7)	0	0	4(50)	0	1(16.7)	11(18)
OX1	34(100)	0	2(40)	4(50)	3(50)	0	43(70.5)
CIP5	17(50)	2(100)	2(40)	0	1(16.7)	3(50)	25(41.0)
PG10	0	0	0	4(50)	0	4(66.7)	8(13.1)
GM10	0	2(100)	0	0	6(100)	3(50)	11(18.1)
VAN10	34(100)	0	2(40)	8(100)	6(100)	1(16.7)	51(83.6)
MER30	12(35.3)	0	3(60)	0	3(50)	2(33.3)	20(32.8)
STX25	2(5.9)	0	1(20.)	5(62.5)	0	0	8(13.1)

Key: Kanamycin (K30), Gentamicin (GM10), Trimethoprim Sulpha (SXT25), Cotrimoxazole (TS25), Oxacillin (OX1), Cloxacillin (OB5), Ampicillin (AMP25), Penicillin G (PG10), Meropenem (MER10), Ertapenem (ETP30), Cefotaxime (CTX30), Chloramphenicol (C30), Ciprofloxacin (CIP5), Vancomycin (VAN10) and Erythromycin (ERY15)

Prevalence of antimicrobial resistance

The AMR profiles of the CNS isolates are summarized in Table 2. All CNS isolates were susceptible to ertapenem. There was high resistance of CNS species towards Vancomycin (83.6%), followed by Oxacillin (70.5%), Cloxacillin (65.6%), Ampicillin (42.6%), Ciprofloxacin (41%), Meropenem (32.8%), Kanamycin (29.5%), Cotrimoxazole (19.7%), Erythromycin (18%), Gentamycin (18%), Cefotaxime (13.1%), Trimethoprim Sulpha (13.1%), Penicillin G (13.1%), and lastly Chloramphenicol (4.9%).

Seven of the CNS species (11.5%) were resistant to at least two antimicrobial drug classes exhibiting some multi-drug resistance (MDR) phenotype as shown in Table 3. All the MDR isolates were resistant to penicillins,

Antimicrobial resistance profiles of different CNS species

Some *S. xylosus, S. epidermidis and S. chromogenes* isolates were each resistant to at most three antimicrobial drug classes showing MDR and the profiles are shown in Table 3. *S. simulans, S. saprophyticus and S. haemolyticus* did not show MDR patterns. *S epidermidis*had the highest MDR proportion which was 14.7%

Table 3. Multidrug resistance profiles of the different CNS species isolated from mastitis milk in Zimbabwe.

CNS spp	Antimicrobial resistance profile	MDR proportion Number of isolates (%)
S. epidermidis	Amp, Ery, Cotri	5(14.71)
	Amp, Cip, Cotri	
	Van, Mer, Ery, Cotri, Pen	
	Van, Ery, Pen	
	Kan, Amp, Ery	
S. xylosus	Van, Ery, Cotri, Pen	1(12.50)
S. chromogenes	Kan, Amp, Chlo, Ery	1(16.67)

Key: Van: Vancomycin, Ery: Erythromycin, Cotri: Cotrimoxazole, Pen: Penicillin G, Amp: Ampicillin, Cip:Ciprofloxacin, Mer: Meropenem, Kan : Kanamycin, Chlo: Chloramphenicol

DISCUSSION

Mastitis is a common disease of economic importance affecting the dairy sector in Zimbabwe and other countries world over. *Staphylococci* bacteria are the major causes of bacterial mastitis in dairy cattle with high incidences of *S. aureus* infections (Bhavana and Chaitanya, 2022). CNS were regarded as minor pathogens but are increasingly reported as the cause of both clinical and subclinical mastitis in dairy cattle (Taponen et al., 2007; Vidlund et al., 2022), with the prognosis in affected cows depending on the antimicrobial resistance profile of the isolated species (Cepas et al., 2019). In this study, we observed a low prevalence of 20.3% of CNS species compared to other studies done in dairy farms around Zimbabwe of 27.6% (Katsande et al., 2013) and 33.2% (Kudinha and Simango, 2002). The slight decrease in prevalence can be attributed to the differences in methodologies (Dego and Vidlund, 2024) or to the fact that most dairy farmers are implementing effective routine mastitis preventative measures such as using individual drying cloths for each cow and regular pre- and post-milking teat dipping. The predominant CNS species was *S. epidermidis* (55.7%) and the least isolated CNS species was *S. haemolyticus* (3.3%). *S. epidermidis* is one of the major Staphylococci commensals on human skin, and most probably originated from the milker's hand (Kudinha and Simango, 2002). However, other studies have shown *S. chromogenes* (Dego and Vidlund, 2024) to be the most prevalent CNS species followed by *S. simulans*, *S. xylosus. S. haemolyticus* and *S. epidermidis* (De Buck *et al*., 2021). It might be concluded that differences in environment and region affect the prevalence and distribution of individual CNS species as well as herd management (Dego and Vidlund, 2024). For example, Farm A had a high prevalence of *S. simulans* (14.3%) whilst farms B, C and E had no *S. simulans* isolates.

All six CNS species showed resistance to at least one antimicrobial. A large proportion of *S. epidermidis* isolates showed high resistance to Vancomycin (100%), Oxacillin (100%) and Cloxacillin (64.7%). Few of these isolates were resistant to Ciprofloxacin (50%), Ampicillin (41.2%), Meropenem (35.3%) and kanamycin (23.5%). The high resistance to oxacillin which is a penicillin is attributed to the fact that it is a very old drug class which has been in use for a long period of time. Its use has been advocated to dairy farmers in Zimbabwe for the intramammary treatment of mastitis as it was previously shown to be effective against CNS species (Kudinha and Simango, 2002). From this study, *S. epidermidis* showed no resistance to ertapenem, penicillin G and gentamycin.

The overall resistance to all 15 antimicrobials by the 61 isolated CNS species was 31%. The highest resistance amongst all the isolated CNS species was against Vancomycin 83.6% (51/61). This was followed by Oxacillin which had 70.5% (43/61), Cloxacillin (65.6%) and Ampicillin (42.66%). Finding vancomycin-resistant CNS in dairy cows poses a major threat to human health, since vancomycin is the antibiotic of choice for the treatment MRSA in humans, stricter measures are needed to curb the use of peptide antibiotics for mastitis treatment in dairy cows. This will help prevent the spread of vancomycin resistance to CNS, potentially safeguarding this critical antibiotic for human medicine. The high resistance of CNS to the penicillins in our study may be a result of PBP2*a* which has a low affinity to the drug. In addition, these antibiotics are readily available over the counter to treat mastitis in dairy cows in Zimbabwe. Because they only target a limited range of bacteria, their overuse might be encouraging the development of resistance in *Staphylococcus* species. In particular, the use of oxacillin under the brand name orbenin® which is used by dairy farmers to treat mastitis intramammarily, could be attributed to the presence of a specific gene (mecA) that mediates oxacillin resistance and a high proportion of β-lactams resistant CNS isolates. Nonetheless, this finding further emphasizes the importance for the careful use of antibiotics in the dairy industry.

Low resistance to chloramphenicol was observed possibly due to the fact that its use in food animals was banned by the World Organization for Animal Health (WOAH). This drug is reported to cause aplastic anemia in humans. Likewise low resistance against ertapenem was seen because they are prescription preparations in Zimbabwe.

According to WOAH, multidrug resistance is typically defined as resistance to three or more antimicrobial agents. The prevalence of MDR CNS species was 11.5% (7/61). The common drug classes amongst the MDR profiles were penicillins and glycopeptides which showed some similarities with quite a number of previous studies thereby suggesting persistence of multidrug CNS species. This is attributed to the affordability, accessibility and availability of these antibiotics in the country as well as their use, misuse and overuse as veterinary medicinal products.

A major limitation to our study was lack of molecular techniques for identification of the different CNS species as well as detection of the resistance genes. Biochemical identification schemes can be used to distinguish the different CNS species but however they can be tedious (Bhavana and Chaitanya, 2022) compared to the molecular techniques which are highly accurate (Matope et al., 2024). Phenotypic methods are continuously implemented especially in low-income countries due to their low cost as well as their simplicity.

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CONCLUSIONS

This study revealed high levels of antimicrobial resistance to Vancomycin, Oxacillin, Cloxacillin, Ampicillin, Ciprofloxacin, varying low resistance to Kanamycin, Meropenem, Cotrimoxazole, Erythromycin, Gentamycin, Cefotaxime, Trimethoprim Sulpha, Penicillin G and Chloramphenicol but no resistance to Ertapenem. The recovery of MDR CNS strains from milk is of great concern as the resistant determinants can spread to the major mastitis pathogens. Future studies should also consider important factors like the breed of the cow, the source of the antibiotics used, whether the farmers have received training on mastitis control and education on proper antibiotic use. The government is therefore recommended to carry out further surveillance of these important pathogens and improve farmer education together with scaling up of the mastitis control programs to cater for CNS species.

ETHICAL APPROVAL

Ethical approval was obtained from the Departmental Board of Clinical Veterinary Sciences.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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