# PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF COAGULASE NEGATIVE STAPHYLOCOCCI FROM PIGS AND HUMANS IN LUSAKA PROVINCE, ZAMBIA

By

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# Copyright Declaration

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# Abstract

Coagulase-negative staphylococci (CoNS) are normal inhabitants of human and animal skin as well as mucous membranes, yet they have emerged as significant pathogens in healthcare settings, causing serious infections. Initially described as avirulent in 1884, CoNS, particularly *Staphylococcus epidermidis*, are now recognized for their role in healthcare-associated infections contributing to 30-40% of nosocomial infections. Recent studies have highlighted the public health implications of CoNS as they can transfer antibiotic resistance genes and cause infections such as urinary tract infections, endocarditis and surgical site infections. While CoNS have been primarily studied in hospital environments, data from community settings, particularly in Africa, remain scarce. This gap in knowledge hinders a comprehensive understanding of the epidemiology and challenges associated with CoNS infections in the region. The objective of this study was to characterize CoNS isolated from pigs and humans working with pigs on selected farms in Lusaka Province using phenotypic and molecular techniques including the prevalence and resistance profiles of CoNS in Zambia. This was a cross-sectional study which involved archived CoNS isolates from pigs and humans working with pigs on selected farms in Lusaka Province. Species identification of the CoNS was done using traditional microbiological techniques such as culture and molecular methods like polymerase chain reaction (PCR). Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method, while data was analyzed using SPSS version 28 (IBM Corp, Armonk, NY, USA). Common species identified were *S. captis, S. warneri, S. xylosus, S. sciuri* and *S. caprae*. The isolates were generally susceptible to antibiotics especially gentamycin (97%), chloramphenicol (96.3%) and ciprofloxacin (93.3%). Despite being susceptible, they also expressed a high prevalence of multidrug resistance at 60.7% to beta-lactams, tetracyclines, phenicols, sulfonamides, lincosamides and aminoglycosides. While, Methicillin Resistant-CoNS were prevalent when tested using phenotypic methods, none of the MR-CoNS tested positive for the *mecA* gene using PCR, suggesting other resistance mechanisms may be at work. These findings are significant to both animal and human health and suggest the importance of increased surveillance, antimicrobial stewardship (AMS) and research into resistance mechanisms of CoNS.

# Dedication

I dedicate this dissertation to my late father, whose invaluable life teachings helped me become the person I am today. Your insight and direction continue to motivate me every day. To my siblings Mutinta, Musowafu and Japhet, may this be a springboard to believe that nothing is impossible to achieve in life.

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# List of Abbreviations

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|---|---|
| CoNS | Coagulase Negative Staphylococci |
| CoPS | Coagulase Positive Staphylococci |
| BHI | Brain Heart Infusion |
| SNP | Single Nucleotide Polymorphism |
| MR-CoNS | Methicillin Resistant Coagulase Negative Staphylococci |
| MDR | Multi Drug Resistance |
| AMR | Antimicrobial Resistance |
| AMS | Antimicrobial Stewardship |
| SSI | Surgical Site Infections |
| FBRI | Foreign Body-related Infections |
| PFGE | Pulsed-Field Gel Electrophoresis |
| WGS | Whole Genome Sequencing |
| OG | Optical density |
| PCR | Polymerase Chain Reaction |
| CRISPR | Clustered Regularly Interspaced Short Palindromic Repeats |
| PIA | Polysaccharide Intercellular Adhesion |
| MALDI-TOF MS | Matrix-Assisted laser desorption/ionization time-of-Flight Mass Spectrometry |
| MGE | Mobile Genetic Elements |
| SCC | Staphylococcal Chromosome Cassette |
| NHRA | National Health Research Authority |
| ACEIDHA | Africa Center of Infectious Diseases for Human and Animals |
| UNZA | The University of Zambia |
| PBP2a | Penicillin Binding Protein 2a |

# CHAPTER ONE

# INTRODUCTION

## 1.0 Background

Coagulase-negative staphylococci (CoNS) are normal inhabitants of animal and human skin as well as their mucous membranes (Mayhall, 2012). They were first described in 1884 by Rodenbach as *Staphylococcus albus*, an avirulent Staphylococcus (Piette and Verschraegen, 2009). CoNS are differentiated from the closely related but more virulent Coagulase Positive Staphylococci (CoPS) like *Staphylococcus aureus* by their inability to produce free coagulase (Rodgers et al., 2009). *Staphylococcus epidermidis* is the most commonly isolated CoNS and ccurrently, there are more than 40 recognized species of CoNS (Archer, 1995; Rogers et al., 2009).

CoNS have long been regarded as harmless skin commensals and dismissed as culture or microbiological contaminants (Hussain, 2011). Recently, CoNS species have piqued public health attention due to their involvement in healthcare-associated infections which have serious consequences for human and animal health (Argemi et al., 2019). Additionally, they are infrequently found in community-acquired illnesses (Abdullahi et al., 2024). They are the most common cause of nosocomial bloodstream infections, responsible for 30% to 40% of these infections (Rupp, 2004). Their ubiquity as commensals and biofilm-forming abilities pose a unique challenge followed by growing concerns about antimicrobial resistance (AMR) within this diverse bacterial group (Bizzini and Dufour, 2012).They have also shown potential to transfer resistant genes to similar organisms and cause infections (Rupp and Archer, 1994). Some of the CoNS species with potential to cause infections include; *S. haemolyticus, S. epidermidis, S. lugdunensis, S. saprophyticus, S. caprae, S. warneri, S. pasteuri, S. capitis, S. sciuri, S. xylosus* and have been associated with urinary tract infections, endocarditis, foreign body related infections, surgical site infections, endophthalmitis and mastitis (Argemi et al., 2019).

CoNS have become increasingly resistant to antibiotics and the most recent threat being the emergence of strains with moderate levels of resistance to vancomycin. They also possess a common theme to readily acquire antibiotic resistance traits, greatly limiting treatment options. This particularly holds true for *S. epidermidis* and *S. haemolyticus* isolates from nosocomial sources where methicillin resistance rates up to 80% are common (Becker et al., 2014). However, in non-healthcare-associated environments such as livestock, antibiotic-resistant staphylococci may also be found, suggesting a putative role for CoNS as reservoirs for the evolution and spread of resistance genes (Xu et al., 2018). Studies of CoNS around the globe have made significant advancements in characterizing CoNS genomes using whole-genome sequencing. They have identified core and accessory genomes across different CoNS species, revealing the presence of virulence factors and genes associated with antibiotic resistance (Becker et al., 2020). This knowledge helps differentiate harmless commensal strains from pathogenic ones, understand the mechanisms by which CoNS cause infection and track transmission patterns within healthcare facilities and communities (Asante et al., 2020). Most of this available literature is well documented in hospital acquired CoNS infections than community acquired CoNS infections (Heilmann et al., 2019).

While global research on CoNS is progressing, data from Africa is limited. Existing research primarily focuses on documenting the prevalence and mechanisms of AMR in CoNS isolated from hospitals, highlighting concerns about antibiotic misuse in African healthcare settings (Asante et al., 2020). There is a scarcity of dedicated research comparing CoNS strains from hospital and community environments in Africa. This lack of data hinders a complete understanding of the epidemiology and challenges associated with CoNS infections on the African continent (Asante et al., 2020). Therefore, more research is needed in Africa to address the continent's specific challenges regarding CoNS infections and AMR.

Currently in Zambia, speciating of CoNS has not been done, hence there is little knowledge about them. This study aims to shed light on the potential health impact of CoNS in the face of antimicrobial resistance. By exploring existing and future avenues through which these bacteria could acquire and disseminate resistance, the study holds the potential to inform targeted interventions and strategies for tackling this evolving public health threat.

## 1.1 Statement of the Problem

CoNS are often misdiagnosed as contaminants due to their commensal nature and lack of specific virulence characteristics compared to *S. aureus*. This underestimation of their clinical significance hinders effective diagnosis, treatment and control of infections in humans and animals. Despite literature revealing that CoNS have the potential to not only cause infections but also to store and pass on resistance genes to other pathogens of similar characteristics especially *S. aureus*, contributing to AMR (de Oliveira et al., 2002) and there are no published studies in Zambia. Also, there is a critical need to define the specific role of CoNS in infections of humans and animals in order to understand their contribution to antibiotic resistance, transfer and identify novel markers for precise differentiation between colonization and infection. Furthermore, a lot of studies done found CoNS but were never studied and focus has been on the hospital associated infections of CoNS than those in the community (Perez et al., 2019). Similarly, a study conducted by Samutela and others in 2023 found a lot of CoNS in the community but no further analysis was conducted (Samutela, 2023).

## 1.2 Rationale of the Study

CoNS are the most prevalent Gram-positive cocci that are commensals to healthy human beings as well as animals. Because of this, they are considered less important when isolated from both humans and animals and this leads to misdiagnosis of infections caused by CoNS and these infections pose a growing threat to human and animal health. Additionally, previous research done highlights CoNS potential not only to cause infections but also act as reservoirs and vectors of antibiotic resistance genes, particularly to *S. aureus* (Otto, 2013). This alarming evidence necessitates a comprehensive scientific investigation into CoNS. This study addresses the unknowns about CoNS especially on antimicrobial susceptibility patterns, resistant genes and provide information on the zoonotic potential of the CoNS isolates thereby safeguarding the future of effective antimicrobial therapy for both humans and animals.

## 1.3 Significance of the Study

CoNS, previously overlooked as harmless, are becoming pathogens of concern in humans and animals. This study aims to address this knowledge gap by investigating their antibiotic resistance patterns, potential as resistance gene reservoirs and zoonotic transmission. By improving CoNS diagnosis, informing antibiotic use and revealing transmission risks, this research has the potential to significantly improve human and animal healthcare. The prevalence of AMR in CoNS is important to consumers of pigs, policy makers and farmers.

## 1.4 Research Questions

1. What species of CoNS are present in pigs and humans working with pigs on selected farms in Lusaka Province?
2. What are the antibiotic susceptibility and resistance patterns in CoNS isolates from pigs compared to those from humans?
3. What resistant genes are found in CoNS from pigs and humans in Lusaka Province?

## 1.5 Objectives

### 1.5.1 General objective

The general objective was to characterize CoNS isolated from pigs and humans working with pigs on selected farms in Lusaka Province.

### 1.5.2 Specific Objectives

1. To identify and speciate the CoNS isolated from pigs and humans working with pigs on selected farms.
2. To determine the antimicrobial susceptibility patterns of the CoNS isolates to commonly used anti-staphylococcal antibiotics.
3. To determine resistant genes in CoNS isolates.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.0 An overview of Staphylococcus species

Staphylococcus belongs to the Staphylococcaceae family, Bacillales order, Bacillus class and Firmicutes phylum. Staphylococci are Gram-positive bacteria that are commonly found on the skin and mucous membranes of humans and animals (Ghebremedhin et al., 2008). Staphylococci are divided into two groups: coagulase-positive (CoPS) and coagulase-negative (CoNS). CoPS have been found to cause infections, whereas CoNS have originally been believed to be safe for humans. *S. aureus* and *S. pseudintermedius* are the most significant pathogens in the CoPS group affecting both humans and animals, especially *S. aureus* (Marsilio et al., 2018). Multi Drug Resistance (MDR) strains of CoNS can cause serious infections, particularly in immunocompromised persons (Piette and Verschraegen, 2009; Otto, 2013). Although pigs are occasionally infected with *S. aureus*, they can harbor methicillin-resistant strains without exhibiting any symptoms (Crombé et al., 2013). The primary concern with Methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs is the potential for human transmission, given that pigs may serve as reservoirs for these strains.

## 2.1 Historical Perspective of CoNS and Microbiology

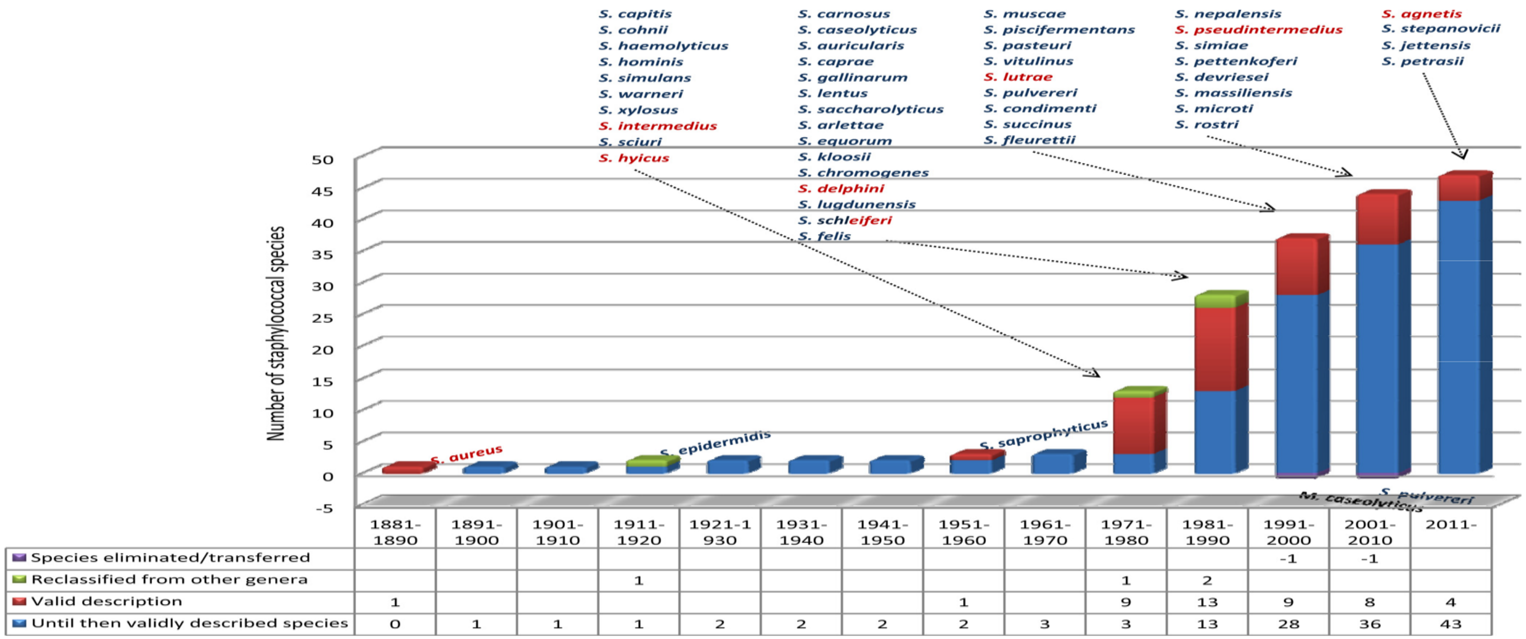
The dualism theory describes the earliest ideas of separation within the Staphylococcus genus. Similar to other genera, numerous taxonomic reclassifications and species renaming occurred during the early stages of the staphylococci discovery. It is important to keep in mind the various conceptions of species and the restricted means of identification that were used in the pre-molecular period when reading through earlier literature. Surgeons such as Billroth who documented "*Coccobacteria septica*" in 1874 and Alexander Ogston who coined the word "Staphylococcus" in 1882, were the pioneers in establishing a direct connection between Staphylococcus-like microbes and wound infections (Becker et al., 2014). In terms of pathogenicity, one of the earliest references to distinct species being named "Micrococcus" and specifically, "Staphylococcus" was provided in 1884 by German surgeon Rosenbach. Rosenbach showed through animal experiments and cultivation that various microorganisms could be recovered from abscesses; these were named

"*Staphylococcus pyogenes albus*" and "*Staphylococcus pyogenes aureus*" (Rosenbach, 1884). Rosenbach's animal tests later proved the pathogenicity of the pus-derived "albus" variety, indicating that it was likely a less or nonpigmented *S. aureus* isolate. However, "*Staphylococcus epidermidis albus*" was identified by American pathologist Welch in 1891 as a nearly constant colonizer of human epidermidis, which was also detected in aseptic wounds (Becker and Peters, 2014).

It was difficult to differentiate between the two pathogenic staphylococcal "varieties" since the temporary division of staphylococci into two genera in the early 1900s (Aurococcus [including *Aurococcus aureus*, linked to diseased tissues] and Albococcus [including the first acceptable taxonomic identification of *S. epidermidis*, as *Albococcus epidermidis*]) (Winslow and Winslow, 1908). This was a common topic in a number of historical scientific publications. Even though this approach was ultimately widely regarded as unsatisfactory, the genus Staphylococcus was initially classified based on the synthesis of pigment in the early decades of research into staphylococcus-like bacteria.

Coagulase production was first identified by R. W. Fairbrother in 1940 as a key factor that distinguished staphylococcal species (Fairbrother, 1940). But rather than referring to it as "*S. epidermidis*," in order to differentiate between nonpathogenic CoNS and CoPS which are known as "*S. pyogenes*," Fairbrother proposed the taxon "*S. saprophyticus"* (Fairbrother, 1940). The designation "*S. saprophyticus*" was later used more broadly by Shaw et al. in 1951, however, the type of strain that these authors initially designated still corresponds to the type strain of *S. saprophyticus subsp. saprophyticus* (Shaw et al., 1951). The inherent capacity of staphylococci and micrococci to ferment glucose in an anaerobic environment allowed for their differentiation. Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) notes that *S. saprophyticus* was reclassified in 1974 after being mistakenly assigned to the subgroup "Micrococcus, subgroup 3" (Maskell, 1974), due to its sluggish fermentation of glucose in an anaerobic environment. With the discovery of 10 newly identified species (such as *S. haemolyticus, S. hominis* and *S. intermedius*) in the 1970s, the era of a small number of staphylococcal species came to an end. By the start of 2014 (Becker et al., 2014), there were more than 40 validly described species, which marked a progressive increase (Figure 1).

Regarding other CoNS, the clinically defined "*S. epidermidis* group" can be distinguished from *S. saprophyticus* by the latter being a specific cause of acute urethritis. This group includes the most common species *S. haemolyticus* as well as other species that have historically been included such as *S. capitis, S. hominis, S. simulans* and *S. warneri, S. saprophyticus*, however, it can also be identified as a pathogen that causes infections similar to those reported in *S. epidermidis* group members. This group may also include some of the recently identified CoNS species, such *S. massiliensis* and *S. pettenkoferi* (Becker et al., 2014).



| | 1881-1890 | 1891-1900 | 1901-1910 | 1911-1920 | 1921-1930 | 1931-1940 | 1941-1950 | 1951-1960 | 1961-1970 | 1971-1980 | 1981-1990 | 1991-2000 | 2001-2010 | 2011- |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 🟪 Species eliminated/transferred | | | | | | | | | | | | -1 | -1 | |
| 🟩 Reclassified from other genera | | | | 1 | | | | | | 1 | 2 | | | |
| 🟥 Valid description | 1 | | | | | | | 1 | | 9 | 13 | 9 | 8 | 4 |
| 🟦 Until then validly described species | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 3 | 3 | 13 | 28 | 36 | 43 |

Figure 1:Time line of the discovery of the species belonging to the genus Staphylococcus (Becker et al., 2014)

Coagulase-negative species are shown in blue; coagulase-positive and coagulase-variable species are shown in red (note that only *S. schleiferi subsp*. coagulans is coagulase positive). Note that at the times of establishment of the first three species designations *S. aureus, S. epidermidis* and *S. saprophyticus*, these terms comprised a broader content than that accepted today. In particular, *S. epidermidis* and *S. saprophyticus* were used to describe non pathogenic, saprophytic staphylococci (and other Gram-positive cocci occurring in clusters) (Becker et al., 2014)

## 2.2 Current status of staphylococcal species and subspecies.

Currently, there are 47 species and 23 officially characterized subspecies in the genus of Staphylococcus. 38 of these are classified as coagulase-negative species, while *S. schleiferi* is one more species that has both a coagulase-positive subspecies (*S. schleiferi* subsp. coagulans) and coagulase-negative subspecies (*S. schleiferi subsp. schleiferi*). *S. jettensis, S. massiliensis, S. petrasii* (including *S. petrasii subsp. petrasii* and *S. petrasii subsp. croceilyticus*) and *S. pettenkoferi* are the most recently identified CoNS species isolated from human clinical specimens (Pantucek et al., 2013; Wolska-Gębarzewska et al., 2024).

## 2.3 Epidemiology of CoNS

The barrier function of the skin offers protection and it is home to a wide variety of bacteria, with CoNS being a commensal of both human and animal mucosal microbiota and skin (Asante et al., 2020). The well-known claim that certain Staphylococcus species favor greater humidity locations like the plantar foot region, axillae and umbilicus has been supported by studies of the skin metagenome (Young et al., 2009). The most often isolated CoNS species in humans are members of the S. epidermidis group, which includes *S. haemolyticus* and *S. epidermidis*. These species are typically found in moist areas of the skin (Asante et al., 2020).

Determining the clinical relevance of CoNS presents a significant difficulty. Also, determining whether retrieved CoNS are contaminants that invade during sample processing or sampling, routine skin or mucous membrane commensals or clinically relevant is a common task for microbiologists and clinicians (Becker et al., 2014). The distinction between pathogenicity and harmlessness can be difficult to make because numerous CoNS species are included in the skin and mucous membrane microbiota. This is because different species use different virulence methods and host defense mechanisms play a major role in this process. In the 1980s, the pathogenicity of CoNS was recognized after it was previously thought to be mostly nonpathogenic, nonetheless, there were insufficiently advanced methods available to identify its molecular processes (Becker et al., 2014). In certain sensitive populations including the elderly, critically ill, immunocompromised, premature babies, long-term hospitalized patients and those with invasive devices the importance of CoNS in generating infections is well acknowledged (Asante et al., 2020). Techniques to reduce contamination are necessary if there is a significant number of CoNS in the samples which could be the result of contamination during sampling. Nonetheless, CoNS need to be evaluated since they can be the real source of the infection. There are differences in commensals' capacity to spread infections (DePasse et al., 2013).

The majority of CoNS samples including clinical samples have poorly defined virulence factors which makes it difficult to determine their pathogenicity. However, the presence of certain genetic markers may help distinguish between potentially virulent and saprophytic/contaminating CoNS strains. For instance, the presence of virulence factors such enterotoxins, toxic shock syndrome toxin 1, or Panton-Valentine leucocidin may be linked to an enhanced level of pathogenicity in CoNS (Asante et al., 2020). The distinct spectrum of diseases that CoNS and *S. aureus* cause indicates that the former has a greater number of virulence factors than the latter (Klibi et al., 2018). It has been discovered that the presence of phenol-soluble modulins in *S. epidermidis* is linked to methicillin resistance, endemicity, invasiveness and persistence in hospital settings and CoNS sepsis (Qin et al., 2017).

In a study comparing community CoNS strains to those from clinical sources that looked into what makes CoNS pathogenic, it was discovered that 25% of the strains produced toxins within an hour that caused hemolysis in 50% of human red blood cells and that after 30 minutes, about 3% of the strains exhibited lytic activity on human polymorphonuclear cells. Using PCR and latex agglutination techniques the *staphylococcal enterotoxin C* gene was identified in 9% of *S. epidermidis* strains. In that study, 68% of hospital-associated CoNS strains possessed at least one virulence marker compared to 32% of community strains (Asante et al., 2020). Extracellular enzyme synthesis allows the bacterium to infect a host by disrupting its tissues and suppressing the immune system. For instance, *S. epidermidis* produces elastase which degrades fibrinogen, albumin and fibronectin. Pathogenic conversion of CoNS was found to have improved *S. simulans* survival in whole blood and replication in distal organs due to increased ability to agglutinate in plasma. CoNS simultaneously possessed the *coa, vwb* and *clfA* genes which encode coagulation and agglutination of vertebrate blood. Therefore, it has been suggested that having these factors could be a way for ordinarily commensal staphylococci to become invasive pathogens (Otto, 2013).

## 2.4 Hospital associated CoNS

CoNS are one of the main factors contributing to nosocomial infections in healthcare settings especially infections involving bloodstreams and indwelling devices. The most commonly isolated species in clinical settings is *Staphylococcus epidermidis* which is usually connected to bloodstream infections caused by catheter use (Widerström et al., 2012). Methicillin-resistant CoNS strains are becoming more common, which makes treatment more difficult and places a heavy financial pressure on healthcare systems (Kloos, 1994).

## 2.5 Community Associated CoNS

CoNS are usually considered harmless skin commensals in communal settings. They may, however, act as opportunistic infections, particularly in those with weakened immune systems. CoNS has been linked to infections like skin and soft tissue infections, highlighting the need for better clinical management and raised awareness (Widerström, 2010).

## 2.6 Livestock Associated CoNS

CoNS also play an important role in veterinary medicine, particularly in livestock. They may be connected to infections that cause animal production to suffer large financial losses. For example, bovine mastitis, which affects milk supply and quality has been linked to *S. xylosus* and *S. epidermidis* (Tedeschi et al., 2009). Food safety and public health are at risk due to the spread of antibiotic-resistant CoNS from livestock to humans (Igbinosa et al., 2016).

## 2.7 AMR in CoNS

One of the main concerns in CoNS is antibiotic resistance and they might also serve as a reservoir for a variety of AMR genes including methicillin resistance genes (Otto, 2013; Bhargava and Zhang, 2014; Osman et al., 2016). According to Osman (2015), methicillin-resistant CoNS strains typically have a multidrug resistance phenotype. CoNS have a relatively high rate of penicillin resistance; as late as 1968, 60% of patients had resistance to penicillin (Corse and Williams, 1968). Approximately 91% of clinical strains today are resistant to penicillin (Koksal et al., 2009). Staphylococci's resistance to methicillin is brought on by the production of Penicillin Binding Protein 2a (PBP2a) which is encoded by the *mecA* gene. *MecA* is found on the staphylococcal cassette chromosome (SCC), a genetic element. There is evidence of horizontal gene transfer of SCC cassettes between staphylococcal species, suggesting that the CoNS may act as a reservoir for the dissemination of resistance genes against *S. aureus* (Becker et al., 2014).

Numerous studies demonstrate a rise in MR-CoNS over time and methicillin or oxacillin resistance in Spain grew drastically in 1996 (to 51%) and reached 61% in 2002 after remaining steady (to 26–34% of CoNS) between 1986 and 1994 (Becker et al., 2014). Other studies also reported a rise in methicillin-resistant CoNS strains in blood from patients with bacteremia. The percentage increased from 38% in 1996 to 68% in 2007 (Koksal et al., 2007) in patients receiving peritoneal dialysis the percentage increased from 19% in 1991 to 74% in 1998 (Zelenitsky et al., 2000), respectively. Methicillin resistance is thought to be present in about 80% of nosocomial isolates and 30–40% of isolates recovered from healthy carriers or community patients (Koziol-Montewka et al., 2006). According to Becker et al. (2014), resistance to ciprofloxacin and erythromycin in Spain's CoNS rose gradually from 1% and 41% in 1986 to 45% and 63% in 2002. Comparable susceptibility results were found in two surveillance investigations conducted in 2006, one in the US and the other in Europe, Asia, Australia and Latin America. The levels of susceptibility to fluoroquinolones, erythromycin, clindamycin, trimethoprim/sulfamethoxazole, gentamicin, teicoplanin and linezolid are 42–47%, 30-34%, 60–61%, 62–59%, 71–58%, 99% and 98–99%, respectively (Jones et al.,2007). High percentages of resistance to other antibiotic classes are present in methicillin-resistant isolates. Methicillin-susceptible strains have substantially lower rates of resistance (Sader et al., 2007).

CoNS were the first species to be identified as having acquired glycopeptide resistance (vancomycin MIC ~ 8 mg/mL, teicoplanin MIC ~ 16 mg/mL). There is diverse manifestation of glycopeptide resistance as seen by the majority of clinical glycopeptides resistant isolates that are susceptible to vancomycin but resistant to teicoplanin (Biavasco et al., 2000). CoNS resistance mechanisms against glycopeptides remain incompletely understood. The majority of reports indicate that the resistance mechanism in CoNS is comparable to the strains of VISA and hetero-VISA (Becker et al., 2014). Exposure to teicoplanin and vancomycin can select a multifactorial expression-related pathway in vitro. Identifying glycopeptide resistance in laboratories presents challenges due to technological factors. Disk diffusion susceptibility testing has a high percentage of false-positive results with clinical investigations showing susceptibility rates of 99-100% (Becker et al., 2014).

Among staphylococcal species, prevalence of resistance varies greatly, *S. haemolyticus* has the highest rates of resistance: 76–96% resistant to oxacillin, 80–90% resistant to erythromycin and 26–29% non-susceptible to teicoplanin (Gatermann et al., 2007). Both *S. hominis* (80%) and *S. epidermidis* (38–81%) have high rates of oxacillin resistance (Gatermann et al., 2007). Three percent of *S. epidermidis* isolates and none of the *S. hominis* isolates are teicoplanin non susceptible. *S. lugdunensis* has nearly uniform sensitivity to the majority of antimicrobials including cephalosporins, macrolides and penicillins (Gatermann et al., 2007).

The CRISPR/CRISPR-associated proteins (Cas) systems are thought to be the primary regulators of horizontal gene transfer. In *S. epidermidis*, CRISPR interference has also been reported to impede plasmid transformation and conjugation (Marraffini and Sontheimer, 2008). Nonetheless, while being present in the genomes of roughly 45% of bacteria, CRISPR/Cas systems exhibit a low abundance in CoNS and 15% of the 122 genomes from 15 species of CoNS included CRISPR/Cas systems. This percentage was especially low in isolates of *S. epidermidis* (9%) and *S. haemolyticus* (3%) (Becker et al., 2020). The low CRISPR abundance in CoNS is consistent with their function as efficient gene reservoirs for both virulence and resistance genes. Recombination between phages and CRISPR-cas spacer sequences has also been demonstrated to potentially facilitate horizontal gene transfer in the case of an SCCmec III-A MRSA strain (Becker et al., 2020). Other related taxa of the Staphylococcaceae family such as macrococci also co-exist with CoNS especially on animal skin and the spontaneous exchange of a multi-resistance plasmid between macrococci and staphylococci has previously been reported. Moreover, *Macrococcus caseolyticus* has been shown to have SCCmec-like regions, but the genome of this species' type strain lacks a CRISPR system (Becker et al., 2020).

## 2.8 Resistant genes in CoNS

CoNS carry several resistant genes responsible for antimicrobial resistance. Important resistance genes are *mecA* and *mecC* that encode changed penicillin binding proteins and cause β-lactam resistance. Other important genes are *blaZ* for β-lactam hydrolysis, *vanA* for glycopeptide resistance and *aac/ant/aph* for aminoglycoside modification. CoNS also encode genes such as *tet(K/M),* to efflux tetracycline or provide ribosomal protection and *erm(A/B/C),* which cause macrolide/lincosamide resistance through methylation of the ribosome (Table 1). The broad multidrug resistance seen in CoNS isolates is a result of these genetic components (Reygaert, 2018).

Table 1: Resistant genes in CoNS. Adapted from Reygaert, 2018.

| **AMR Gene** | **Antimicrobial Class: Antimicrobial Agent** | **Mechanism of resistance** |
|---|---|---|
| ***mecA***<br>***mecC*** | β -lactams: Penicillins, Cephalosporins | Altered drug target, Penicillin binding protein 2a(PBP2A) |
| ***blaZ*** | Monobactams, Carbapenems | Hydrolysis of β-lactam antibiotics by β-lactamase. |
| ***vanA*** | Glycopeptides | Vancomycin Resistant CoNS- Modified target |
| ***mprF*** | Lipopeptides: Daptomycin | Change in cell membrane charge-decreased drug binding. |
| ***aac, ant aph*** | Aminoglycosides: Amikacin, Gentamycin, Tobramycin | Aminoglycosides modifying enzymes modify target |
| ***tet (K)***<br>***tet(M)*** | Tetracyclines: Tetracycline, Minocycline, Tigecycline | Active efflux pumping<br>Ribosomal protection by competitive binding |
| ***cat*** | Chloramphenicol | Inactivation by acetylation of the drug |
| ***erm (A)***<br>***erm(B)***<br>***erm(C)*** | Macrolides, Lincosamides: Erythromycin, Clindamycin | Methylation of ribosome to decrease binding |
| ***rm, cfr*** | Oxazolidinones: Linezolid | Mutation of ribosome, methylation of ribosome |
| ***erm (A)***<br>***erm(B)***<br>***erm C)*** | Streptogramins: Quinupristin/ DAlfopristin | Methylation of ribosome |
| ***gyrA***<br>***grlA*** | Fluoroquinoloones: Ciprofloxacin | Gyrase modified target<br>Topoisomerase IV modified target |
| ***norA*** | Norfloxacin, Gatifloxacin, Levofloxacin Moxifloxaxin | Active efflux |
| ***dhfr/ dhps*** | Metabolic pathway inhibitors: Trimethoprim/Sulfamethoxazole | Target enzyme modification |

## 2.9 Molecular Basis of resistance to beta lactam antibiotics and genetics of methicillin resistance

The primary cause of methicillin resistance is the acquisition of genes that code for distinct penicillin-binding proteins (PBP2′ or PBP2a). Even in the presence of penicillins, cephalosporins and carbapenems, PBP2a catalyzes efficient cell wall production with a lower affinity for β-lactam antibiotics. Staphylococcal chromosome cassette mec (SCCmec), is a mobile element that carries the *mecA* gene that encodes PBP2a (Chambers, 1997). SCCmec is a mobile genetic element that consists of two genetic elements: mec, which is in responsible for methicillin resistance and the *ccr* complex which controls the integration and removal of the cassette from the bacterial genome. This mechanism mediates methicillin resistance in strains of Staphylococcus spp. SCCmec I, II, III, IV and V are the five distinct kinds that have so far been identified (Martins et al, 2007).

Ito et al. (2004) provided a detailed description of the first three categories in 2001. The most current type known as type V was reported by the same authors in 2004. The type IV strains were characterized by Ma et al. (2002) and are primarily community-acquired. Compared to types IV and V, SCCmec types I, II and III are noticeably bigger and primarily cause nosocomial infections. Of the three varieties, SCCmec type I is the smallest with a size of 34,364 bp. This cassette lacks any transposon or plasmid that imparts resistance to heavy metals or medications other than methicillin. Nonetheless, a subtype designated as IA has been found that varies from type I in that it carries an integrated plasmid (*pUB110*) (Martins et al., 2007).

The second cassette known as SCCmec type II is 53,017 bp in length and contains transposon *Tn554* which gives this type of isolate resistance to erythromycin and streptomycin in addition to the *mecA* and *mecRI* genes that confer resistance to methicillin. A subtype of this cassette known as IIA exists and it is marginally smaller than type II (40 kb) (Shore et al., 2005). The largest of the five types is SCCmec type III which is 66,896 bp in size and contains the transposons *Tn554* and *ψTn554,* plasmid *pT181*, the *mecA* and *mecRI* genes and transposon *ψTn554* which confers resistance to tetracycline and mercury and plasmid *pT181*, which confers resistance to cadmium.

Apart from the previously mentioned accomplishments, Ito et al. (2004) also observed variations in the ccr gene types with SCCmec type III containing a gene termed *ψccr* that is absent from the other kinds. This result raises the possibility that a few of the type III resistance features could be employed as markers for selection. There are two varieties of this sort of cassette: IIIB lacks copies of both *Tn554* and *pT181* and IIIA lacks both plasmid *pT181* and its surrounding *IS431* element (Shore et al., 2005). The majority of infections acquired in the community are caused by SCCmec type IV. The element is tiny and only carries the *mecA* resistance gene. Furthermore, the cassette exhibits several subtypes, indicating a high transmissibility of SCCmec type IV (22). The sequences of the four SCC type IV subtypes—IVA, IVB, IVC, and IVD—vary upstream of the CCR complex or the L-C region (Shore et al., 2005).

Ito et al. (2004) identified type V, the most recent SCCmec type to be discovered, in an Australian strain. With a size of 27,624 bp, this new cassette is smaller than SCCmec type V but slightly larger than SCCmec type IV. Similar to type IV, SCCmec type V exclusively carries genes that encode methicillin resistance. However, type V differs from the other elements in this family in that it carries a novel form of the *ccr* gene known as type *c* which is present in a single copy as opposed to the other elements' two copies. Methicillin resistance in CoNS is caused by the same methods as those for *S. aureus*, however, *mecA* gene-mediated resistance is often present at lower levels than in MRSA, thus making it harder to detect (Livermore, 2000).

## 2.10 Virulence factors of CoNS and pathogenesis

The "slime" production in CoNS was identified as a significant contributor to the pathogenesis of infections as early as 1972 (Huebner and Goldmann, 1999). For *S. epidermidis,* virulence solely depends on this "slime," or biofilm. The bacterial attachment and persistence on foreign materials are made feasible by the creation of biofilms. In addition, bacteria arranged in biofilms are shielded from the immune system and antibiotics (Mack et al., 2005).

The capacity to form an extremely organized and resilient biofilm on the surface of indwelling medical devices is the most significant virulence factor of CoNS. Three distinct steps are thought to be involved in the creation of biofilms. First, nonspecific forces like hydrophobicity and polarity cause the cells to adhere to the device's surface in a reversible way. Subsequently, the formation of adhesins which bind to human host proteins specifically and coat intravascular catheters and other devices fast leads to more specific adhesion. After being firmly attached, the cells create a thick biofilm that in the case of *S. epidermidis*, is primarily composed of polysaccharide intercellular adhesion (PIA). This milieu leads to antibiotic tolerance and host defense evasion. PIA is produced by the ica ADBC operon in *S. epidermidis* and the production of biofilm in this species requires a functional ica locus (Mack et al., 2005). The gradual, challenging-to-treat nature of these infections is caused at least in part by the presence of this sticky material. Research indicates that the ica locus is more commonly present in *S. epidermidis* isolates obtained from healthcare settings than in isolates from the general public (John and Harvin, 2007). The *ica* operon's genetic control and the creation of biofilms are complex. Table 2 also lists other virulence factors.

Table 2: List of other virulent factors (Rogers et al., 2009).

| Virulence factor | Mechanism | Gene(s) |
|---|---|---|
| **Biofilm** | Immune System Avoidance, Antimicrobial tolerance | |
| **PIA** | Polysaccharide component | *icaADBC* |
| **Aap** | Accumulation of biofilm cells | *aap* |
| **Bhp** | Accumulation | *bhp* |
| **DNA** | Structure of biofilm, nutrient, horizontal gene transfer | *NA* |
| **Adhesin Molecules** | Adherence to Host Proteins or Biomaterials | |
| **Aae** | Binds Fgn, Vn, Fn | *aae* |
| **AtlE** | Binds Vn | *atlE* |
| **Bhp** | Binds polystyrene | *bhp* |
| **Ebp** | Binds elastin | *ebp* |
| **EmbP** | Binds fibronectin | *empB* |
| **Fbe(SdrG)** | Binds fibrinogen | *sdrG* |
| **GehD** | Binds collagen | *gehD* |
| **ScaA(Aae)** | Binds Fgn, Vn, Fn | *scaA* |
| **ScaB** | Unknown ligand | *scaB* |
| **SdrF** | Binds collagen | *sdrF* |
| **SdrG(Fbe)** | Binds fibrinogen | *sdrG* |
| **Ssp-1** | Binds polystyrene | *Unknown* |
| **Ssp-2** | Binds polystyrene | *Unknown* |
| **Teichoic acid** | Binds fibronectin | *tagF, yibD* |
| **Other virulence factors** | Various mechanisms | |
| **Peptidoglycan/** | Stimulates cytokine production | *tagF, femA others* |
| **lipoteichoic acid** | Induction of cytokines, | |
| **Phenol-soluble modulins** | Immune system modulation | *psma, psmb* |
| | Biofilm dispersion | |
| | Immune system avoidance | |
| **Poly-D-glutamic acid** | Aid resistance of antimicrobial | *psmd, psmg* |
| | peptides. | *cap locus* |
| **Delta-toxin Exoenzymes** | Injury to host cell | *hld* |
| **Fatty-acid modifying** | Inactivates fatty acids on skin | *unknown* |
| **enzyme** | Skin and wound colonization | *gehC, gehD* |
| **Lipases** | Destruction of host tissue | *sspA, sspB* |
| **Proteases** | | *sspC* |

**Abbreviations:**

**Aap**, accumulation-associated protein; **Aae**, autolysin/adhesion; AtlE, autolysin; **Bhp**, Bap homolog protein; **Ebp,** elastin-binding protein S; **EmbP**: extracellular matrix-binding protein; **Fbe(SdrG),** fibrinogen-binding protein; **Fgn,** fibrinogen; **Fn**, fibronectin; **GehD,** glycerol ester hydrolase; **PIA**, polysaccharide intercellular adhesion; ScaA**(Aae),** staphylococcal conserved antigen A; **ScaB,** staphylococcal conserved antigen B; **SdrF,** serine aspartate repeats protein F; **SdrG(Fbe),** serine aspartate repeats protein G; **Ssp-1**, staphylococcal surface protein 1; **Ssp-2**, staphylococcal surface protein 2; **Vn**, vitronectin (Rogers et al., 2009).

## 2.11 Plasmids and Transposons

Transposons and plasmids are mobile genetic elements that help bacteria transfer genes that provide virulence and resistance to antibiotics (Alharbi et al., 2024). Through conjugation, bacteria can exchange plasmids which are tiny, self-replicating circular DNA molecules that carry auxiliary genes such as those for virulence and antibiotic resistance. Transposons, on the other hand, are tiny, movable DNA segments that may move between chromosomes and plasmids and carry genes for virulence and antibiotic resistance (Helinski, 2022). Plasmids and transposons facilitate horizontal gene transfer, which allows bacteria to exchange resistance and virulence factors (Gyles and Boerlin, 2014). This leads to the emergence of pathogenic and antibiotic-resistant bacterial strains. Several plasmids are present in most staphylococci, some of which can be passed between species through conjugation (e.g., *S. aureus* or other coagulase negative staphylococci). This appears to be a key mechanism in the dissemination of determinants of antibiotic resistance, particularly beta-lactam and aminoglycoside resistance. In CoNS, transposons have the ability to transfer resistance genes between plasmids and from plasmids to chromosomal locations (Huebner et al., 1999).

## 2.12 Surface Proteins

Many staphylococci cell wall proteins have been identified and it has been found that these proteins specifically bind to extracellular matrix components such as collagen, fibrinogen, fibronectin, vitronectin and laminin (Wilkinson, 1997). It is yet unclear how significant these protein interactions are for the pathophysiology of coagulase-negative staphylococcal colonization or infection. Electron microscopy has identified a fimbria-like protein structure that may be involved in the attachment of CoNS to foreign materials in the host. A 140-kD extracellular protein is also suggested for the accumulation of *S. epidermidis* on surfaces. An uncharacterized hemagglutinin has been associated with adherence to polymer surfaces. In contrast, *S. saprophyticus* infections are linked to the attachment of hemagglutinin and surface fibrillar proteins to urinary tract epithelium, with invasion attributed to a urease (Huebner et al., 1999).

## 2.15 Specific Infections

### 2.15.1 Urinary Tract Infections (UTIs)

After *Escherichia coli, S. saprophyticus* is the second most common causal bacterium of uncomplicated UTI in females. Although rare, complications have been reported including recurring infections, acute pyelonephritis, nephrolithiasis, septicemia and endocarditis (Widerstro¨m, 2010). *S. saprophyticus* can be pathogenic at low counts (<105 cfu/mL) (Rupp and Archer, 1994). Since bacteriuria is typically seen as insignificant particularly in cases when bacterial counts are low, *S. saprophyticus* is most likely frequently missed as the causal organism of UTIs. Additionally, 7% of rectal swabs obtained from pig and bovine carcasses have been shown to have *S. saprophyticus*. The microbe is frequently present in a variety of dietary samples but it is more prevalent in raw beef and pig. The gastrointestinal system is *S. saprophyticus* main human reservoir (Rupp and Archer, 1994). There are rare instances where other CoNS species mainly *S. epidermidis* are identified as the cause of UTI (less than 5% of UTIs in hospital settings). *S. epidermidis* is typically isolated from elderly hospital patients who use urinary catheters or who undergo other procedures that manipulate the urinary tract (Rupp and Archer, 1994).

### 2.15.2 Endocarditis

Despite being rare, prosthetic valve endocarditis (PVE) is often caused by CoNS. 15% to 40% of PVE cases are diagnosed with PVE caused by CoNS (typically *S. epidermidis*). *S. epidermidis* is the primary cause of CoNS Native Valve Endocarditis (NVE) (85%). *S. hominis* (6%), *S. lugdunensis* (5%), *S. capitis, S. caprae* and *S. simulans* are responsible for the remaining fraction. Patients with CoNS and *S. aureus* NVE were shown to have comparable rates of heart failure and death (Chu et al., 2004). Similar to patients with viridans streptococcal NVE, patients with staphylococci-caused NVE had a much higher risk of endocarditis related to medical treatment. *S. lugdunensis* causes a variety of diseases, similar to *S. aureus* (Heilbronner and Foster, 2020) and it is particularly more virulent than other CoNS and has been linked to potentially fatal infective endocarditis (Aldman et al., 2021). The infections caused by *S. lugdunensis* are quite severe with majority of the cases are acquired in the community and only a small percentage of patients roughly, 25%, have replacement valves (Rupp and Archer, 1994).

### 2.15.3 Mastitis

Human mastitis is rarely linked to the CoNS. S. aureus and CoNS are the agents most commonly cultured from milk in lactation mastitis. CoNS infections are not the only pathogen found in non-lactational breast abscesses. There have been some documented occurrences of non-lactational breast abscesses in literature, particularly with *S. lugdunensis.* The most frequent microorganisms to cause breast abscesses are anaerobes and *S. aureus* (Rupp and Archer, 1994).

### 2.15.4 Surgical site infection (SSI)

*S. aureus* is the most often isolated bacteria from surgical site infections (SSI) (20%), followed by CoNS (14%) and Enterococcus species (12%) (CDC,1996). After *S. aureus* (21%) and Streptococcus species (11.2%), CoNS are the third (10%) most commonly isolated microorganism in a secondary care hospital (Piette and Verschraegen, 2009). Although there have been documented SSI outbreaks that started with operating room staff, the majority of infections are most likely indigenous (Huebner and Goldmann, 1999). After median sternotomy, the CoNS is primarily responsible for causing sternal osteomyelitis (Rupp and Archer, 1994).

### 2.15.5 Catheter-Related Infections

Bacteremia caused by CoNS is most frequently caused by infections associated to catheters. About 50–70% of these infections are caused by *S. epidermidis*. Due to its high strain variability, *S. epidermidis* is the most prevalent CoNS responsible for a wide range of clinical disorders such as prosthetic device-associated infections and neonatal sepsis (Severn and Horswill., 2019). Within medical/surgical intensive care units (ICUs), the rate of device-associated infection for central line-associated bloodstream infections is between 4.5 and 14.6 per 1000 central line days (Eggimann and Pittet, 2000). Approximately 50% of all hospitalized patients are given an intravascular catheter during their stay. Bacteremia rates are lowest (0.04 per 100 catheter days) for fully implanted catheters (e.g., Port-A-Cath), and lowest (0.14 per 100 catheter days) for long-term Hickman or Broviac catheters. As much as 30% of these catheters will eventually develop an infection mainly due to their frequently extended use (Herrmann and Peters, 2005). Life-threatening CoNS bacteremia is rare especially when treated quickly but sepsis can occur, particularly in patients with weakened immune systems. Infected thrombophlebitis, exit site infections and tunnel infections can all be caused by CoNS. There have been reports of other problems including abscesses and infective endocarditis (Herrmann and Peters, 2005).

### 2.16 Methods of Detections of CoNS

As a general rule, it's critical to differentiate between CoPS and CoNS, this is typically done in laboratories using the slide and tube coagulase tests. Accurate clinical decision making by clinicians is facilitated by species-level identification especially for species that are suspected of causing illnesses. In nosocomial settings in particular, accurate CoNS speciation is crucial for identifying the precise sources of infection. Reliable, affordable and quick identification techniques are necessary for accurate diagnosis and timely treatment. Biochemical testing, including kits that are sold commercially are the primary means of achieving this. Correct identification by biochemical tests is typically difficult since different CoNS species exhibit comparable biochemical features and species heterogeneity. Also, biochemical techniques are time-consuming (Pfaller and Herwaldt, 1988).

Molecular techniques like polymerase chain reaction (PCR) and spectrometric techniques like matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) are gradually replacing speciation with biochemical techniques. Therefore, suitable molecular techniques that provide a higher degree of identification precision up to the species and subspecies level are recommended. However, when low-quality sequence data are published into databases, sequence data analysis problems may arise when using molecular approaches that entail sequence data analysis (De Almeida et al., 2018).

The majority of the time species are reported as a general group and identification is not done which restricts the amount of information that can be found regarding their epidemiology and minimizes the true impact of the different especially nonabundant species. The identification approach MALDI-TOF MS has been demonstrated to have good accuracy and reproducibility when used to identify CoNS and it is also beneficial in toxin detection (De Almeida et al., 2018). The rapid turnaround times and inexpensive operational costs of MALDI-TOF MS are among its other advantages. Many laboratories particularly in low- and middle-income nations cannot afford the instrument's initial cost due to its high cost. Additionally, it has been found that in nearly 50% of cases, MALDI-TOF is unable to detect uncommon species (Abd El-Aziz et al., 2021). It has also been demonstrated that with differing degrees of discriminatory power, target housekeeping genes like the elongation factor *Tu (tuf)* gene, *superoxide dismutase (sodA)*, *RNA polymerase B (rpoB)* and *16S rDNA* gene can help distinguish between Staphylococcus species and subspecies. In order to achieve greater precision and reproducibility in CoNS speciation, molecular techniques like PCR are advised (Abd El-Aziz et al., 2021).

## 2.17 Typing Methods of CoNS

Studies on clonal diversity and relatedness in CoNS have received less attention than those on *S. aureus*. CoNS species including *S. lugdunensis, S. haemolyticus* and *S. schleiferi* are not as clonally varied as *S. aureus* which is distinguished by a broad genetic diversity according to pulsed-field gel electrophoresis (PFGE) data. For the purpose of characterizing MR-CoNS clones in epidemiological research, SCCmec typing is crucial (Asante et al., 2020). Using SCCmec typing Klibi et al. (2018) identified methicillin-resistant *S. epidermidis* to have SCCmec type-Iva while examining the characteristics of methicillin resistant and susceptible CoNS collected from cow milk samples. Additionally, PCR analysis of the *ccr* recombinases revealed that the isolates carried the recombinase genes *ccr A2/B2-class B mec* gene complex (Asante et al., 2020).

A study conducted in Tunisia reported SCCmec types I, II, IV, and V/VII. The study also discovered a variety of PFGE patterns in *mecA*-positive CoNS isolates. Similar SCCmec and pulsed field gel electrophoresis (PFGE) patterns of MR-CoNS were seen in that investigation across several sources indicating the transmission and circulation of the same clones at the interface between humans, animals and the environment. The fact that only a small number of studies conducted genetic typing even when CoNS were the main pathogens suspected of causing infection indicates that CoNS genetic typing has not received the same attention as *S. aureus* and other pathogens. As a result, identifying the most prevalent clones or varieties in use across the continent is challenging. Microbiologists have benefited greatly from the combination of the aforementioned techniques although their capacity to distinguish between bacterial strains that originated from a single bacterial clone is restricted (Harris et al. 2012). While whole genome sequencing (WGS) may identify novel kinds or subtypes and detect single alterations in the genomes of two isolates, it is therefore an appropriate epidemiological typing technique. Efficient epidemiological typing can help, for instance, by connecting virulence factors with resistance in hospital-endemic CoNS clones. High-throughput sequencing methods are becoming more affordable but they are still not a common tool used in regular epidemiological investigations in resource-constrained environments like most of Africa (Asante et al., 2020).

## 2.18 Treatment and Control of CoNS

Recognizing that many CoNS are methicillin resistant, there are few available treatment choices. As a result, the glycopeptides in particular, vancomycin are essential especially for treating infections caused by *S. epidermidis* species (Asante et al., 2020). Penicillin, first- or second-generation cephalosporins and isolates that are methicillin-sensitive can all be employed. However, in cases when methicillin resistance is discovered, cotrimoxazole or more recent generation antibiotics such as daptomycin and linezolid may be used (Becker et al., 2014). Treatment plans for infections connected to invasive devices should be customized in light of the devices' function. UTIs linked to catheter use are among them. In these situations, the removal of the devices might be explored, however, this would require further medical procedures and costs. In certain cases, removing the device may be sufficient to reduce infection. Antimicrobial therapy should be administered for 10–14 days if devices are kept. It is important to take into account the susceptibility patterns of CoNS while designing both empirical and targeted treatments (Becker et al., 2014).

## 2.19 The Economic Impact, Public Health and Interactions

CoNS have a significant economic impact especially in healthcare settings where they have been linked to a number of infections. Even though CoNS are regarded to be harmless skin commensals, there is growing recognition of their role as opportunistic infections, particularly in patients with implanted medical devices and immunocompromised patients. A number of factors contribute to the financial burden of CoNS infections, such as longer hospital admissions, more expensive medications needed to treat resistant strains and more diagnostic testing required (Skoglund et al., 2019). According to a study by Juthani-Mehta. (2014), hospitals may lose up to $200,000 a year treating CoNS bacteremia needlessly because contaminants were mistakenly diagnosed as actual infections (Juthani-Mehta et al., 2014). Also, the economic effects go beyond the direct expenditures of healthcare as CoNS infections can raise morbidity and mortality which can then have an influence on worker productivity and the nation's total economic output. These financial difficulties are made worse by the high frequency of antibiotic resistance among CoNS strains. The treatment of infections produced by these organisms frequently necessitates the use of last-resort medicines which are much more expensive, due to methicillin resistance rates as high as 70–80% (Waltzman and Harper, 2001). This resistance raises the expense of healthcare and prolongs hospital stays in addition to complicating treatment plans.

In addition to being pathogens of concern in human medicine, CoNS also cause a number of illnesses in animals especially in the production of livestock which results in financial losses. CoNS are well-known primary cause of mastitis worldwide especially in Europe. Once thought to be opportunistic pathogens, they are responsible for mild mastitis that is typically asymptomatic. However, as CoNS species are regularly identified from the majority of intramammary infections (IMIs), their significance in IMIs is growing (Zadoks et al., 2011).

## 2.20 Impact of CoNS on Human Disease

Out of the 41 CoNS species (as of July 2018), only a few have been consistently linked to infections in humans. These organisms which inhabit various cutaneous and mucosal niches in humans and animals frequently have unique virulence factors and can survive on synthetic surfaces when foreign bodies are implanted (Figure 2). There are notable changes in CoNS pathogenicity between species and even strains; *S. haemolyticus* and *S. epidermidis* for instance, have the potential to be medium-pathogenic but *S. carnosus* and other species are wholly apathogenic. The distinction between pathogenic and nonpathogenic CoNS depends on the host immune system's state as it affects both the onset and course of CoNS disease (Heilmann et al., 2019). According to a thorough examination of the composition of the human nasal culture, *S. epidermidis* colonizes almost every person (97%) followed by *S. haemolyticus* (44%), *S. hominis* and *S. capitis* (each 41%), *S. warneri* (32%) and *S. lugdunensis* (26%), (Kasper et al., 2016). These species also comprise the clinically most common CoNS and are frequently compiled and referred to as the "*S. epidermidis* group," with the exception of *S. lugdunensis*. Additional clinically significant CoNS species include

*S. lugdunensis* and *S. saprophyticus subsp. saprophyticus* (Raz et al., 2005). *S. epidermidis* is the primary participant among the CoNS of the *S. epidermidis* group, which are by far the most frequently recovered microorganisms from various types of temporarily or permanently placed foreign bodies followed by *S. haemolyticus, S. hominis* and *S. saprophyticus* (Heilmann et al., 2019) (Figure 2).

Sub-acute and persistent infection courses with mild clinical signs describe the majority of infections caused by this group (Becker et al., 2014). However, it has been documented that patients with persistent foreign body-related infections (FBRIs) who are not well managed may experience more aggressive courses and abruptly fatal outcomes (Heilmann et al., 2019). Significantly longer hospital and intensive care unit stays, greater death rates and higher hospital expenses have all been linked to FBRIs of the bloodstream by CoNS. Increased death is a result of delayed detection of CoNS illnesses (Frickmann et al., 2018). In cases where the infection is bloodstream related, CoNS of the *S. epidermidis* group produce systemic FBRIs with the risk of septic thrombophlebitis, embolic consequences and metastatic seeding at the appropriate insertion sites (Becker et al., 2014). An underdeveloped immune system or immunosuppression are additional risk factors for CoNS infections unrelated to external bodies. Particularly, invasive CoNS infections such sepsis, endocarditis, meningitis and FBRIs are more common in preterm infants, which increases hospital stay duration, morbidity and death. CoNS belonging to this group are the primary cause of septicemia in neutropenic individuals (Horasan et al., 2011).

Recently, several new species of CoNS were found in clinical samples including *S. massiliensis, S. pettenkoferi* and *S. petrasii*. Their significance is now unknown; rather than new pathogenic potential, discovery of these species is probably the consequence of enhanced diagnostic testing (Spanu et al., 2011).

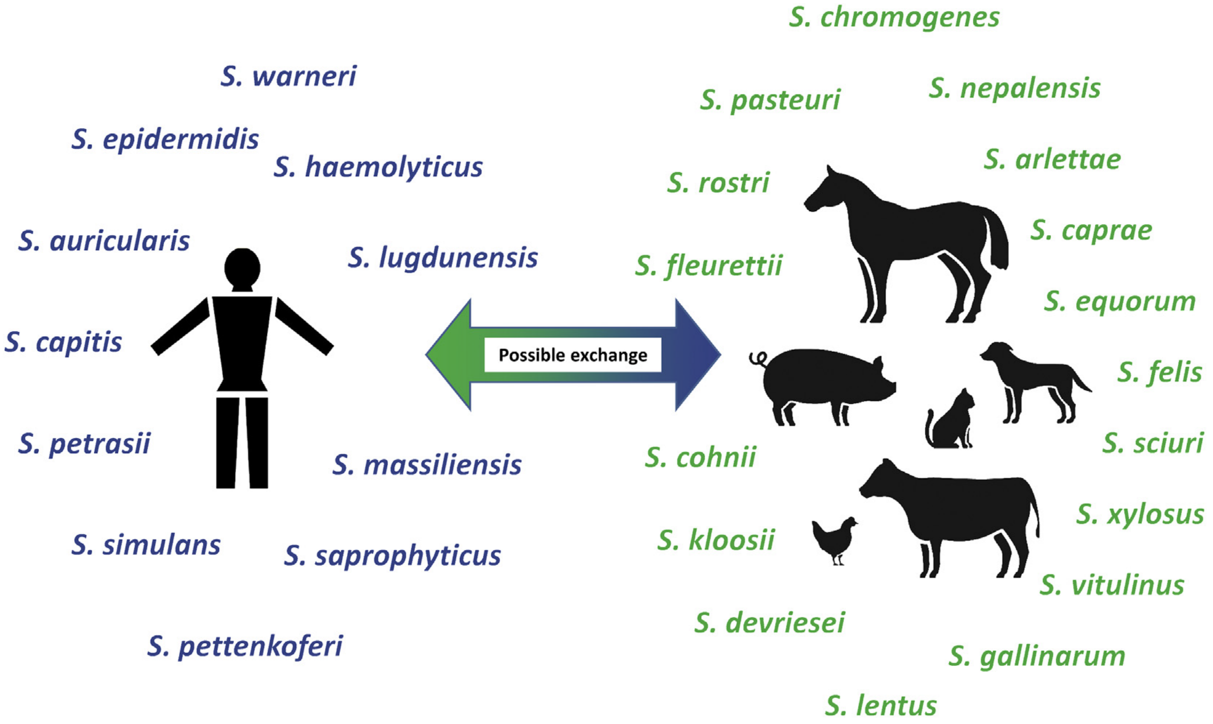


Figure 2: In general, CoNS species are obtained from specimens of humans (left) and farm and companion animals (right). (Heilmann et al., 2019).

Numerous CoNS species that are largely linked with humans are also frequently isolated from companion and farm animals. CoNS, or staphylococci that don't coagulate (Heilmann et al., 2019).

### 2.21 Impact of CoNS in The Livestock Environment and Veterinary Medicine

Similar to humans, CoNS infect warm-blooded animals (Figure 2). It is uncommon for animals to have a change in the host-guest equilibrium that favors opportunistic CoNS infections. As a result, reports of clinical cases are uncommon and unreliable and the clinical importance of CoNS in veterinary medicine is significantly reduced (Heilmann et al., 2019). The cause of CoNS-induced bovine mastitis is currently under investigation; nevertheless, breeding-induced udder anatomy may provide an exception. Acute and persistent clinical infections of the mammary gland and teat canal have been linked to CoNS. Nonetheless, those infections typically don't manifest or are rather minor (Walker et al., 2011).

One component that is poorly understood is the role of human and especially animal-associated CoNS as a potential reservoir for resistance. The same might hold true for other members of the Staphylococcaceae family, like macrococci, in which reports of the transfer of a plasmid-borne methicillin resistance gene to *S. aureus* have been reported (Heilmann et al., 2019). Extremely high resistance rates, especially in *S. sciuri,* were found in a pig farm investigation (Schoenfelder et al., 2017). The majority of resistance traits were found in different CoNS and were found to be linked with multi resistance genes found on mobile genetic elements, indicating horizontal gene transfer between species. As a result, the role that environmental and animal-associated CoNS may have in the establishment and spread of antibiotic and heavy metal resistance genes into more pathogenic species, including *S. aureus*, is of significant concern because it can facilitate the emergence of multidrug-resistant strains that pose a greater threat to public health and limit treatment options (Schoenfelder et al., 2017).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.0 Study Design

This was a cross-sectional study on archived uncharacterized CoNS isolated from pigs and workers from selected pig farms in Lusaka Province in Zambia particularly Lusaka, Chilanga and Chongwe districts conduced between July 2024 to November 2024.The archived samples were collected between June 2020 to July 2022 from a previous study (Samutela, 2023). These isolates were stored in 20% glycerol at -80 °C at The University of Zambia, School of Veterinary Medicine, Microbiology Laboratory in the Department of Paraclinical Studies.

## 3.1 Study Site

The archived CoNS isolates were collected from selected commercial and small-scale pig farms in Lusaka province of Zambia. Lusaka province is the smallest province in Zambia covering an area of 21,896 km$^2$. It also hosts the capital city of Zambia, which serves as both the province's and the nation's hub. Despite the fact that Lusaka province is divided into eight districts, farms in the following districts were chosen for sampling in this study; Lusaka, Chilanga and Chongwe (Figure 3) due to the concentration of commercial pig farms in these areas.

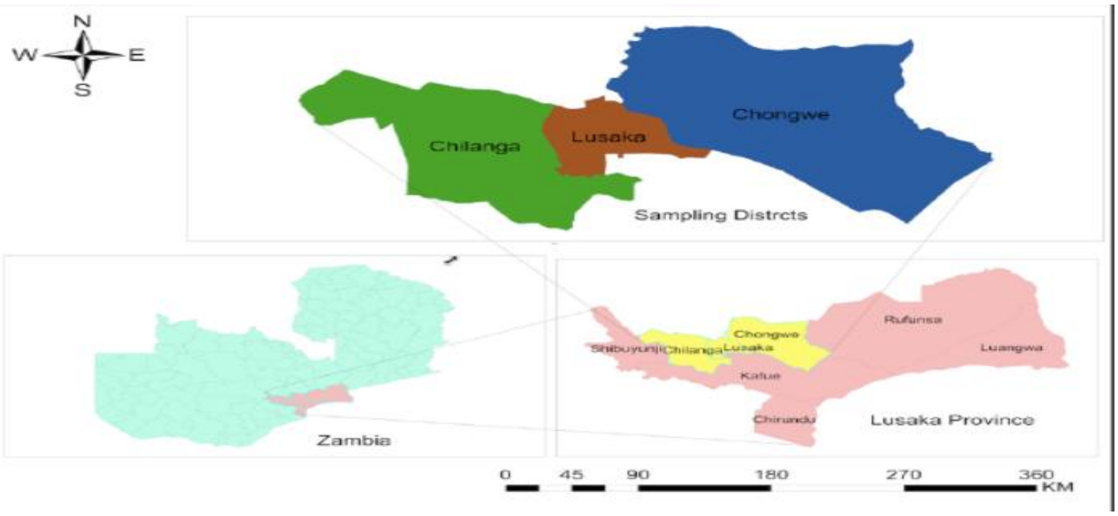


Figure 3: Map of Study Site Showing the Selected Districts of Lusaka Province, Zambia (Samutela, 2023).

## 3.2 Sampling Frame and Sample Collection

Farm owners were asked to sign a consent form granting permission for the study to be conducted on their farm. The target group for the study included workers who had close contact with the pigs at a particular farm as well as pigs of all ages. Pigs' nasal swabs were obtained by gently rotating a dry swab into each of their two anterior nares.Farm employees who had regular interaction with the pigs were asked if they w ould be  interested in taking part in the research. All workers who signed the consent form gave their consent and samples were collected by gently spinning a sterile, dry swab in each of their anterior nares. After that, another sterile swab was used to rub each worker's palms. This allowed for the collection of nasal and hand swabs from all consenting workers. This came once the study's goal was explained in terms that the farm owners and employees could comprehend. The swab samples were then placed in Amies transport media and kept at 4°C in a cool box with ice packs. After that, they were sent to the University of Zambia's School of Veterinary Medicine Microbiology lab for culture (Samutela, 2023).

## 3.3 Inclusion and Exclusion Criteria

### 3.3.1 Inclusion Criteria

The study covered farms situated in the chosen districts of the province of Lusaka. Pig farm owners, farm workers who were at least 18 years old and granted their consent to participate in the study were included as participants.

### 3.3.2 Exclusion criteria

Since it was previously determined that a minimum of 18 pigs per farm was needed to establish the desired prevalence and precision based on a two-stage calculation of sample size adapted from (Van Lochem et al., 2018), farms handling fewer than 20 pigs were excluded from the study.

## 3.4 Sample Size

Purposively, 135 pig and human nasal swabs were obtained from 13 farms from a previous study, 129 from pigs and 6 from humans (Samutela, 2023). The farms were divided into the following districts according to the size of pig farming: two medium-sized farms in Chilanga, one commercial farm and four medium-sized farms in

Chongwe district and two medium-sized farms and four commercial farms in Lusaka district.

## 3.5 Detection of CoNS in Pigs and Humans in Lusaka

### 3.5.1 Phenotypic Detection of CoNS

Standard microbiological techniques were utilized to find and classify CoNS in the samples (Ugwu et al., 2015). Stored samples were removed from the freezer, thawed and then inoculated into an enrichment media namely Brain Heart Infusion Broth (BHIB) (Oxoid, Basingstoke, UK) for 16 to 20 hours at 37°C. The enriched samples were then sub-cultured onto Blood Agar. Pure colonies were subjected to Catalase and Coagulase tests using 3% Hydrogen perioxide (Merck-KGaA, Darmstadt, Germany) and rabbit plasma (Sigma-Aldrich, Taufkirchen, Germany), respectively. The tube coagulase test utilizing rabbit plasma was set up in accordance with manufacturer's instructions (Sigma-Aldrich, Taufkirchen, Germany) using 0.5 ml of the BHIB culture. The test was read every 30 minutes for four hours and then again after 24 hours of incubation at 37°C. All isolates that tested negative for coagulase were identified as CoNS. The test was conducted alongside a known in house positive control *S. aureus.* Therefore, the presumptive diagnosis for CoNS from the archived samples was their inability to clot rabbit plasma.

### 3.5.2 Molecular Confirmation of CoNS Isolates

To definitively identify the species using molecular techniques, the following steps were employed.

#### 3.5.2.1 DNA Extraction

In order to perform DNA extraction, pure CoNS isolates were recovered and the crude DNA extraction procedure was used to extract DNA. Briefly, blood agar was used to grow bacteria overnight. Next, three to five colonies of a pure culture were put into a 1.5 mL Eppendorf tube containing 200 μL of sterile molecular grade water (Nuclease free water). The bacteria combination was then vortexed briefly before being heated in a heating block for approximately 15 minutes at 95°C. The mixture was centrifuged for two minutes at 13000 xg and the extracted DNA aliquots (supernatant) were stored at -30°C until they were subjected to further analysis. All PCR analyses that followed used this DNA. The amount of DNA extracted was estimated using a NanoDrop spectrophotometer. An optical density (OD) of 1 at 260 nm corresponded to a DNA concentration of 50µg/ml of double-stranded DNA and a DNA/protein absorbance ratio of 260 nm/280 nm. The bacterial DNA was kept in the refrigerator at -30°C until they were needed.

#### 3.5.2.2 CoNS Species Confirmation by Polymerase Chain Reaction (PCR)

The following specific primers for the CoNS species were used; Staphylococci (general), *S. saprophyticus, S. epidermidis, S. xylosus, S. warneri, S. haemolyticus, S. lugdunensis, S. pasteuri, S. caprae, S. Capitis* and *S. sciuri* as listed in Table 3. PCR was conducted to confirm and speciate CoNS. A final PCR volume of 12µl was used consisting of 1µl of the bacterial DNA template, 0.5µl of each primer, 6.25µl of the OneTaq Quick-Load 2X PCR Master Mix, and 3.75µl of nuclease-free water (Thermo Scientific, Hanover, MD, USA). The Veriti 9600 Well thermal cycler (Applied Biosystems, CA, USA) was used to perform PCR amplifications. The amplified DNA fragments were observed under a UV light box by comparing the gels stained with ethidium bromide (Cinnagen Co., Tehran, Iran) with a molecular size marker (100 bp ladders, eurobio, UK). The PCR products were electrophoresed in 1.5% agarose gels with 1X Tris-acetate-EDTA buffer at 100V for 30 minutes.

Table 3: Primers of different CoNS species (Adegoke and Okoh, 2014)

| Target Microorganism | Primer | Sequence (5'-3') | Amplicon Size |
|---|---|---|---|
| ***S. xylosus*** | *XylF* | AACGCGCAACGTGATAAAATTAATG | 539 |
| | *XylR* | AACGCGCAACAGCAATTACG | |
| ***S. pasteuri*** | *PA237F* | GCTAATTTAGACAGTGTACCTTCTG | 237 |
| | *PA237R* | GCCCGTTATTTACTACTAACCA | |
| ***S. warneri*** | *SwarF* | TGTAGCTAACTTAGATAGTGTTCCTTCT | 63 |
| | *SwarR* | CCGCCACCGTTATTTCTT | |
| ***S. haemolyticus*** | *ShaeF* | GTTGAGGGAACAGAT | 85 |
| | *ShaeR* | CAGCTGTTTGAATATCTT | |
| ***S. caprae*** | *CR252F* | AATTTAGATAGCGTACCTTTG | 252 |
| | *CR252R* | AGTTACGATTTCTAATTGACCGTT | |
| ***S. epidermidis*** | *Se705-1* | ATCAAAAAGTTGGCGAACCTTTTCA | 1124 |
| | *Se705-2* | AAAAGAGCGTGGAGAAAAGTATCA | |
| ***S. capitis*** | *Scap F* | GCTAATTTAGATAGCGTACCTTCA | 208 |
| | *Scap R* | CAGATCCAAAGCGTGCA | |
| ***S. saprophyticus*** | *Sap1F* | TCAAAAAGTTTTCTAAAAAATTTAC | 221 |
| | *Sap2R* | ACGGGCGTCCACAAAATCAATAGGA | |
| ***S.lugdunensis*** | *F* | CAAAATGTATTAAATGCTAT | 421 |
| | *R* | ATTTAATGATATTTCCTTGAT | |
| ***S. sciuri*** | *SSCGF* | GAT TCC GCG TAA ACG GTA GAG | 306 |
| | *SSCGR* | CAT CAT TTA ATA CTT TAG CCA TTG GA | |
| **Staphylococci** | *TStaG422F* | GGC CGT GTT GAA CGT GGT CAAATCA | 370 |
| | *TStag765R* | TIA CCATTT CAG TAC CTT CTG GTA | |

According to the primers, the PCR conditions for different species were as follows: For *Xyl* gene (*S. xylosus*), *Ssci (S. sciuri), Se705 (S. epidermidis)* and *PA237 (S. pasteuri)* the cycle conditions were as follows: a 4-minute initial denaturation step at 94°C; 30-cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute; and a 4-minute final extension step at 72°C. *Tstag422* gene (Staphylococci) was set up at; a 4-minute initial denaturation step at 94°C; 30-cycles of 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 1 minute; and a 4-minute final extension step at 72°C. *Slug*;4-minute initial denaturation step at 94°C; 30-cycles of 94°C for 30 seconds, 44°C for 30 seconds and 72°C for 1 minute; and a 4-minute final extension step at 72°C while *Sap (S. saprophyticus), Swar (S. warneri), Scap (S. captis)* and *CR252 (S. caprae)* at a 4-minute initial denaturation step at 94°C; 30-cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 minute; and a 4-minute final extension step at 72°C.

Since there are over 40 species of CoNS, speciating all of them was not possible in this study and only those that were associated with infections were speciated. Additionally, speciation was done for the 20 isolates that were phenotypically identified as Methicillin Resistant Coagulase Negative Staphylococci (MR-CoNS).

## 3.6 Determination of Antimicrobial Susceptibility and Resistance Genes in the CoNS Isolates

### 3.6.1 Phenotypic Determination of Methicillin Resistance and Susceptibility to Other Antibiotics

The standard cefoxitin antibiotic discs were used to identify methicillin resistance (CLSI, 2020). The M100 Clinical and Laboratory Standard Institute (CLSI, 2020) states that the disc diffusion test should be used to determine the antibiotic susceptibility of the CoNS isolates. Cefoxitin is a more powerful inducer of *mecA* than penicillins, making it a potential surrogate marker for oxacillin resistance detection (Rostami, 2013). Penicillin (10 µg), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2µg), gentamicin (10 µg), tetracycline (30 µg), trimethoprim/sulphamethoxazole (25 µg), chloramphenicol (30 µg), oxacillin (1 µg) and ciprofloxacin (5 µg ) are the antibiotics that were utilized.

### 3.6.2 Detection of Methicillin Resistance Genes

Using primers for the *mecA* gene (Table 4), PCR was used to determine if the isolates had methicillin resistance genes, following earlier techniques (Milheiriço et al., 2007; Stegger et al., 2012). These tests were conducted using previously published PCR protocols with gene-specific primers (Sutcliffe et al., 1996, Aarestrup et al., 2000). A final volume of 12µl was used, consisting of 1µl of the bacterial DNA template, 0.5µl of each primer, 6.25µl of the OneTaq Quick-Load 2X PCR Master Mix, and 3.75µl of nuclease-free water (Thermo Scientific, Hanover, MD, USA). The Veriti 9600 Well thermal cycler (Applied Biosystems, CA, USA) was used to perform PCR amplifications. For the *mecA* gene, the cycle conditions were as follows: a 4-minute initial denaturation step at 94°C; 30-cycles of 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 1 minute; and a 4-minute final extension step at 72°C. The amplified DNA fragments were observed under a UV light box by comparing the gels stained with ethidium bromide (Cinnagen Co., Tehran, Iran) with a molecular size marker (100 bp ladders, eurobio, UK). The PCR products were electrophoresed in 1.5% agarose gels with 1X Tris-acetate-EDTA buffer at 100V for 30 minutes.

Table 4: Primer Sets for Determining Antimicrobial Resistance Gene (mecA) in CoNS Isolates (Stegger et al.,2012).

| **Primer name** | **Target gene** | **Primer Sequence (5'-3')** | **Amplicon size** |
|---|---|---|---|
| ***mecA (F)***<br>***mecA (R)*** | *mecA* | TCCAgATTACAACTTCACCAg<br>CCACTTCATATCTTgTAACg | 162bp |

### 3.7 Data Analysis

The study's data was analysed using SPSS version 28 (IBM Corporation, Armonk, NY, USA). Univariate analysis was performed on CoNS found in sample categories. Results were given as frequencies and percentages with 95% confidence intervals in graphs. The PCR gel images were analyzed to identify species, resistance and virulence genes by comparing amplicon sizes to expected band sizes.

### 3.8 Ethical Considerations

Isolates from a previous study were used in this study, therefore, ethical waiver and authority to conduct the study was obtained from the University of Zambia Biomedical

Research Ethics Committee (UNZABREC) (Appendix A) and National Health Research Authority (NHRA) (Appendix B).

# CHAPTER FOUR

# RESULTS

## 4.0 Identification of CoNS species

Phenotypic methods yielded 135 bacterial isolates, all of which were confirmed as CoNS using PCR. Of the 135 isolates; 95% were from pigs and 5% from humans. Figure 4 shows results of Gel Electrophoresis of the *Tstag* gene which is found in all Staphylococci species.

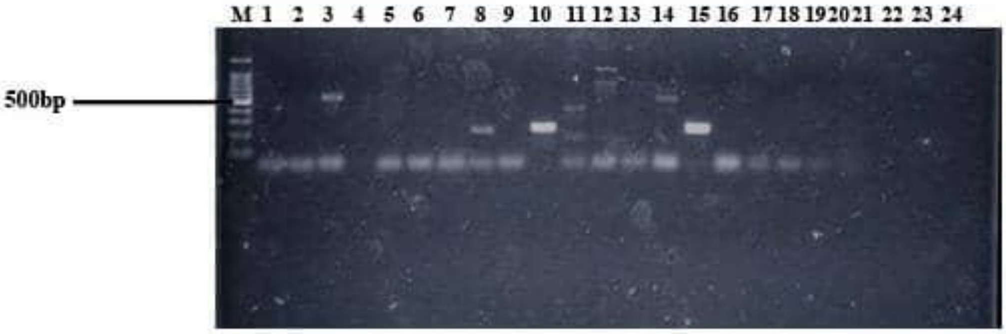


Figure 4: Representative Gel electrophoresis of *Tstag* gene of the CoNS Isolates.

Key: MM = 100bp marker; Lane 1= Negative control; Lane 8 = Positive control; Lanes 10 and 15 = Some of the Samples

The common species identified were *S. capitis* (5%, 1/20), *S. warneri* (10%, 2/20*), S. xylosus* (5%, 1/20), *S. sciuri* (10%, 2/20) and *S. caprae* (5%, 1/20). Among the identified species, only 5% of *S. warneri* were from humans and the rest of the species were from pigs.

## 4.1 Antibiotic Sensitivity Profiles of the CoNS

### 4.1.1 Prevalence of AMR in CoNS

All the 135 isolates were tested and exhibited resistance to beta-lactam antibiotics (Figure 5). Notably, 20 isolates were phenotypically resistant to cefoxitin (19.30%), suggestive of MR-CoNS, oxacillin (100%) and penicillin (99.7%).

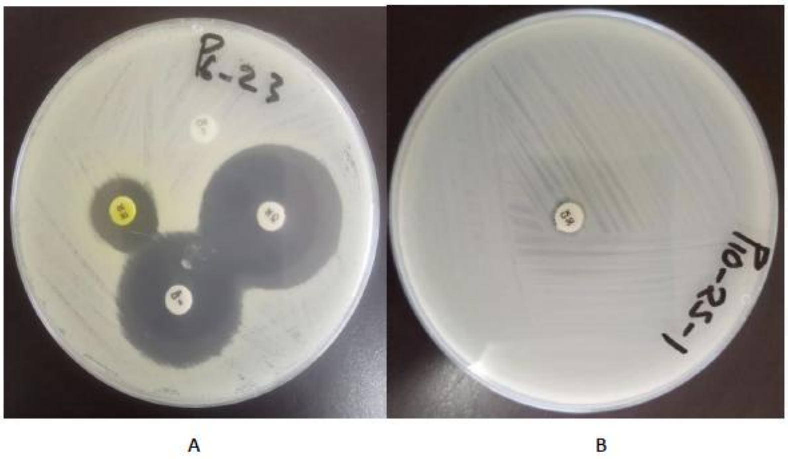


Figure 5: Susceptibility to Beta-lactam Antibiotics (A) Susceptible isolates and (B) Resistant Isolates.

Generally, the isolates were susceptible to the antibiotics tested in the study, with the highest susceptibility being to gentamycin (97%), chloramphenicol (96.3%) and ciprofloxacin (93.3%) and lowest susceptibility to tetracycline (27.4%), cotrimoxazole (17%) and clindamycin (20.7%) as shown in Figure 6.

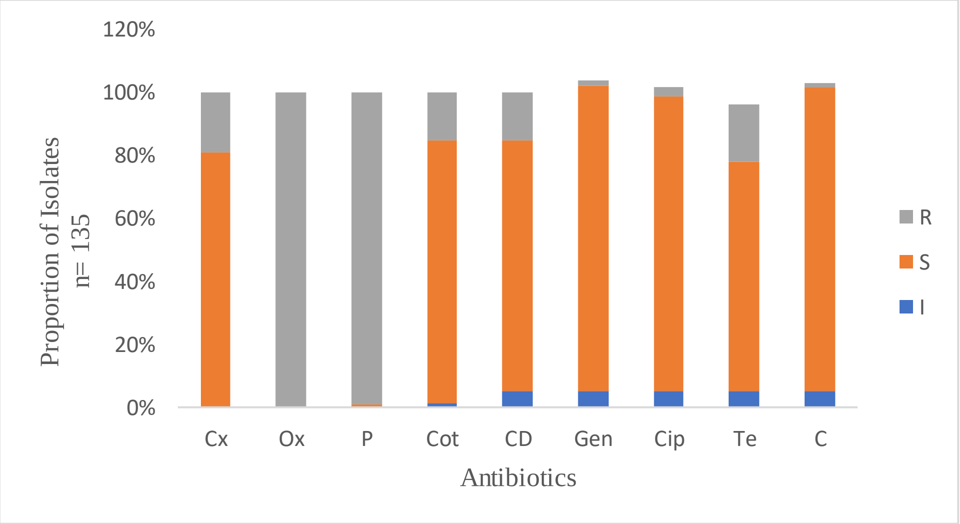


Figure 6: Overall Antimicrobial Susceptibility Frequencies of CoNS Isolates from Pigs and Workers from farms of Lusaka.

**Abbreviations:** P = Penicillin; Gen = Gentamicin; CD = Clindamycin; Cip = Ciprofloxacin; Te = Tetracycline, Cot= Cotrimoxazole, C= Chloramphenicol, CX= Cefoxitin; Ox= Oxacillin I = Intermediate, R = Resistant, S = Susceptible.

### 4.1.2 Multidrug resistance patterns in CoNS isolates.

To determine whether antibiotic resistance phenotypes grouped together, antibiotic resistance patterns were assigned using designations P + Ox + Te + Gen + CX + CD +

Cip + C + Cot. As indicated in Table 3, the isolates categorized into 21 patterns of antibiotic resistance. The majority of the isolates exhibited resistance to at least two or more antibiotics other than penicillin and oxacillin (100%); P + Ox + Te (14.07%) and P+ Ox + Cot + Te (8.89%) were the most common phenotypes. If an isolate exhibited resistance to three distinct classes of antibiotics, it was categorized as multidrug resistant (MDR) (Abdullahi, 2023). Eighty-two (60.7%) isolates were classified as MDR and were resistant to three or more antibiotics.

Table 5: Antibiotic Resistance Patterns of CoNS Isolates from Pigs and pig farm workers in Lusaka Province's Pig Farms.

| | **Resistance Pattern** | **Proportion of Isolates %(n)** |
|---|---|---|
| | | **Farm Isolates (n = 135)** |
| **1** | P + Ox | 100(135) |
| **2** | P + Ox + Cot + CD | 7.40 (10) |
| **3** | P + Ox + Gen + Te | 3.0(4) |
| **4** | P + Ox + CX | 5.20(7) |
| **5** | P + Ox + CX + CD | 2.22(3) |
| **6** | P + Ox + Cot + Te | 8.89(12) |
| **7** | P + Ox + CD | 5.93(8) |
| **8** | P + Ox + Cip | 3.70(5) |
| **9** | P + Ox + Te | 14.07(19) |
| **10** | P + Ox + Cot + CD + Cip | 3.70(5) |
| **11** | P + Ox + Cot + CD | 3.0(4) |
| **12** | P + Ox + CX + Cot + Te | 5.93(8) |
| **13** | P + Ox + CD + Te | 3.70(5) |
| **14** | P + Ox + CX + Cot | 1.48(2) |
| **15** | P + Ox + CX + Gen + CD | 3.70(5) |
| **16** | P + Ox + CX + Gen + CD + Te | 6.67(9) |
| **17** | P + Ox + CX + Te | 1.48(2) |
| **18** | P + Ox + Te + C | 5.93(8) |
| **19** | P + Ox + CX + Cot + Cip + Te + C | 3.70(5) |
| **20** | P + Ox + CX + CD + Te + C | 5.20(7) |
| **21** | P + Ox + CX + Cip | 0.74(1) |

**Abbreviations:** n = number of samples; P = Penicillin; CN = Gentamicin; CD = Clindamycin; Cip = Ciprofloxacin; Te = Tetracycline, Cot= Cotrimoxazole, C= Chloramphenicol, CX= Cefoxitin; Ox= Oxacillin.Each pattern was unique to each isolate.

### 4.1.3 Resistance Pattern of Phenotypically identified MR-CoNS

Some of the MR-CoNS were among the isolates that expressed MDR and were resistant to at least three antibiotics with the highest resistance seen in the combination of P + Ox + CX + Cot + Te (4/20, 2.96%). The sequence of decreasing resistance pattern was P + Ox + CX + Gen + CD + Te (2/20, 1.48%), P + Ox + CX + CD + Te + C (2/20, 1.48%), P + Ox + CX + Gen + CD (1/20, 0.74%), P + Ox + CX + Cot + Cip + Te + C (1/20, 0.74) (Figure 7).

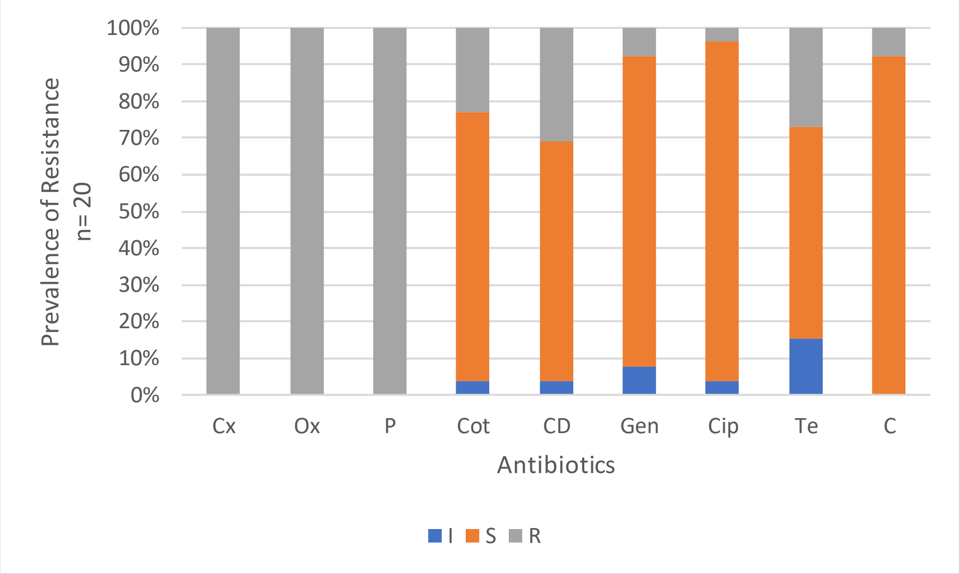


Figure 7: Resistance Patterns in Phenotypically identified MR-CoNS.

**Abbreviations:** P = Penicillin; Gen = Gentamicin; CD = Clindamycin; Cip = Ciprofloxacin; Te = Tetracycline, Cot= Cotrimoxazole, C= Chloramphenicol, CX= Cefoxitin; Ox= Oxacillin I = Intermediate, R = Resistant, S = Susceptible.

### 4.2 Methicillin Resistant Genes in CoNS

The *mecA* gene was not detected in any of the 20 isolates that were phenotypically identified as MR-CoNS.

# CHAPTER FIVE

# DISCUSSION AND CONCLUSION

## 5.0 Discussion

Coagulase-negative staphylococci constitute a large group of micro-organisms that commonly occur on the skin and mucous membranes of humans and animals. Although these species were previously considered as non-pathogenic organisms, some have currently emerged as important opportunistic pathogens, especially in immune-deficient individuals and in nosocomial infections (Cunha et al., 2004). Species identification of CoNS is important for their epidemiology and potential pathogenicity. The aim of the present study was to characterize CoNS isolated from pigs and humans working with pigs on selected farms in Lusaka Province using molecular techniques to determine the prevalence, species and resistance profiles of CoNS in Zambia.

The current study successfully confirmed the species of the 135 CoNS isolates that were obtained from humans and pigs in Lusaka Province by molecular analysis. The most commonly identified species in the study was *S. capitis*, followed by *S. warneri, S. xylosus, S. sciuri* and *S. caprae*. This finding is in agreement with a study by Bonvegna et al. (2021) who reported a high presence of *S. sciuri* in pigs. Other earlier studies also found *S. sciuri* to be the most predominant organism isolated in pigs alongside other species like *S. warneri, S. xylosus* and *S. caprae* (Schoenfelder et al., 2017). Since *S. sciuri* is also known to induce lethal exudative epidermitis in piglets (Cheng et al., 2007), which is typically brought on by *S. hyicus*, which produces exfoliative toxins, the high percentage of *S. sciuri* in pigs is particularly significant. The adaptability of *S. sciuri* to various ecological niches may be the reason for its predominance in the current study. In addition to its wide host range, *S. sciuri* is thought to be able to live freely, which would account for its frequent detection from dust and other materials (Nemeghaire et al., 2014). *S. capitis,S. warneri, S. xylosus, S. sciuri* and *S. caprae* are all associated with various infections, including neonatal sepsis, endocarditis, osteomyelitis, mastitis, skin and soft tissue infections, UTIs, bone and joint infections respectively (Rupp and Archer, 1994; Heath et al., 2023). While majority of research on the CoNS detected in our study has been done on surgical site infections in humans, little is known about them in animals, particularly pigs, which may limit our understanding of the significance of the majority of these species. However, bacteremia in people and mastitis in animals have been commonly linked to the majority of these CoNS species (El-Jakee et al., 2013). Despite the low zoonotic potential of some of these CoNS species, they should be closely monitored to prevent antibiotic resistance which may in turn limit therapeutic choices for treating infections caused by them in humans and animals.

The antibiotic susceptibility profile of the isolates demonstrated varying levels of resistance. The isolates showed the highest sensitivity to gentamicin, chloramphenicol and ciprofloxacin at 97%, 96.3% and 93.3%, respectively. This is in line with a study by Gizaw et al. (2020) that found 96.4% of the CoNS farm isolates sensitive to both gentamycin and ciprofloxacin. Contrary to these findings, another study found that gentamycin resistance was higher (18.4%) (Ma et al., 2011). This variation may be caused by the usage, distribution or prescription of ciprofloxacin, chloramphenicol and gentamycin (Gizaw et al., 2020). Due to a lack of literature about the sensitivity of CoNS isolates in Zambia to chloramphenicol, gentamicin and ciprofloxacin, the usage of these antibiotics in veterinary practice appears to be low hence the high sensitivity. Additionally, they are more reserved for usage in humans than animals in Zambia.

Although the isolates expressed high sensitivity to the above mentioned three antibiotics, they were also seen to be resistant to beta-lactam antibiotics including Penicillin which was at 99.9%, this was higher than 87.1% resistance to Penicillin which was found by Kirwa et al. (2022) in CoNS isolated from camels' fresh milk. Other countries that have reported high resistance of CoNS in both animals and humans to penicillins include; Pakistan (77.8%), China (94.2 %) (Syed et al., 2018) and Tunisia (70.6%) (Klibi et al., 2018). The majority of the isolates in our study expressed resistance to two or more antibiotics in addition to beta-lactams. This might be related to the fact that CoNS are frequently described as co-resistant to several types of antibiotics including methicillin (Huber et al., 2011). The primary cause of resistance in these antibiotics is their initial empirical therapy use as first line antibiotics without proper dosage or withdrawal period consideration (Omwenga et al., 2021). Farmers frequently utilize beta-lactam antibiotics in the management of a variety of diseases especially diarrheal infections which piglets are prone to. This wide use of antibiotics as well as self-medication of animals by the farmers often subjects the animals to sub therapeutic doses and this may be the cause of this significant high resistance to penicillins (Manandhar et al., 2021). The problem can be avoided by limiting antibiotic use to veterinarians and also by offering health education to the communities of Lusaka where these farms are found on the role of antimicrobial resistance to their health and wellbeing. Other antibiotics to which the isolates showed resistance to were cefoxitin (19.30%), tetracycline (18.50%), cotrimoxazole and clindamycin (15.50%), ciprofloxacin (3%) and chloramphenicol (1.50%). This was slightly similar to another study conducted by Kirwa et al. (2022) which found resistance to cefoxitin (10.7%), ciprofloxacin (4.1%), tetracycline (3.3%) and chloramphenicol (1.6%).

Tetracycline was among the antibiotics to which the isolates relatively had high resistance compared to others. Consistent with the current study is a study conducted by Adegoke and Okoh (2014) which found 83.3% CoNS resistant to tetracyclines in farm animals. This can be attributed to the use of tetracycline as a "growth promoter" or "feed efficiency product" that is added to feed at subtherapeutic levels in addition to using it to treat animal diseases in order to enable them gain weight while providing the same quantity of food (Chopra and Roberts, 2001). It also has bacteriostatic properties which reduce bacterial cell multiplication rather than cause bacterial cell death. Additionally, tetracyclines exhibit antagonistic actions with penicillins and cephalosporins, which may eliminate the effects of penicillins and cephalosporins (Lenart-Boroń et al., 2016).

The findings of the present study demonstrate the presence of multidrug-resistant CoNS, which were isolated from pigs and humans from farms in Lusaka Province. About 60.70% of the isolates were categorized as multidrug-resistant (MDR), this was higher compared to the 30.5% MDR found in Staphylococci isolates from camels' nasal cavities in Samburu, Nakuru and Isiolo in Kenya by Mutua et al. (2017). Combinations like Penicillin + Oxacillin + Tetracycline and Penicillin + Oxacillin + Cotrimoxazole were among the most prevalent resistance patterns in the present study. The usage of antibiotics has caused bacteria to select for resistance to microbial agents (GARP, 2011). This can occur through the transfer of resistance plasmids or through inherent resistance exhibited by microorganisms (Kirwa, 2022). Notably, this rising multidrug resistance in pigs can be ascribed to easy access to antimicrobials over the counter, farmers' self-medication of their pigs and overuse, including overdose or underdosing (Lamuka et al., 2017). Due to the possibility of zoonotic transmission, this outcome is dangerous not only for the health of the animals but also to the general public. The multidrug-resistant characteristics from the isolates highlight the need for continuous monitoring and efficient antimicrobial stewardship strategies.

In the present study, CoNS isolates resistance to cefoxitin was at 19.30%. This was less than 60.70% observed by Gizaw et al. (2020). Despite phenotypic methods detecting MR-CoNS, molecular techniques, however, were unable to detect the *mecA* gene in the MR-CoNS isolates, indicating that their resistance phenotype might be influenced by additional genetic factors (Soares, 2012). These findings agree with another study by Kulangara et al. (2017) who found MR-CoNS phenotypically that tested negative for *mecA* gene. The absence of the *mecA* gene in the phenotypically identified MR-CoNS suggests that the resistance observed phenotypically may be caused by other mechanisms such as the production of a new methicillinase, a change in the penicillin binding protein or increased production of beta lactamases encoded by the *blaZ* gene (Dos Santos et al., 2016; Soares, 2012) or presence of uncharacterized β-lactam resistance determinants in the CoNS. This disparity further highlights how intricate AMR mechanisms are in the CoNS and the need of sophisticated molecular methods to completely describe these microorganisms. Another study by Nemeghaire et al. (2014) found that a close homologue of the *mecA* gene, which does not confer resistance to β-lactam antibiotics, has been found in staphylococcal species that belong to the *S. sciuri group,* including *S. sciuri, S. fleurettii, S. lentus, S. stepanovicii* and *S. vitulinus*.

In this study, patterns of antibiotic resistance in CoNS may have significant effects on the environment, public health and the treatment of human and animal diseases. The potential for cross-species transmission of resistant bacteria complicates the treatment of human infections (Hernando-Amado et al., 2019). Pigs and humans with antibiotic-resistant CoNS are more likely to contract infections that are more difficult to cure which can result in longer hospital stays and greater medical expenses (Dadgostar, 2019). The problem of antibiotic resistance may worsen due to the spread of resistance genes within the community, making it more difficult to control bacterial infections.

Manure and wastewater runoff can contaminate the environment, spreading bacteria resistant to antibiotics. This could upset natural microbial communities and have an impact on ecosystem functions (Jian et al., 2021). Because antibiotic resistance is common in veterinary medicine, more cautious use of antibiotics and alternative treatment approaches are required. These findings highlight the critical need for coordinated efforts to address antibiotic resistance in both human and animal populations using the one health approach.

## 5.1 Limitations of the Study

Financial limitations prevented this study from speciating all the isolates and thoroughly screening all possible resistance genes in CoNS, which may have resulted in an underestimation of the scope of antimicrobial resistance mechanisms found in the isolates.

# CHAPTER SIX

# CONCLUSION AND RECOMMENDATIONS

## 6.0 Conclusion

1. The prevalence, identification and patterns of AMR of CoNS isolated from humans and pigs in Lusaka Province were investigated in this study and 135 isolates in all were identified as CoNS, with the most common species being *S. capitis, S. warneri, S. sciuri, S. xylosus* and *S. caprae*.
2. The results showed that the isolates were generally susceptible to antibiotics and methicillin-resistant CoNS (MR-CoNS) was quite common and they were particularly resistant to penicillin and oxacillin.
3. The identification of multidrug-resistant patterns in over half of the isolates raises considerable concern about the consequences for both animal and public health, even though the majority of isolates showed susceptibility to gentamicin, chloramphenicol and ciprofloxacin.
4. The study also demonstrated the intricacy of resistance mechanisms, namely the absence of the *mecA* gene in phenotypically MR-CoNS isolates, indicating alternate resistance routes which underscores the need for more research.

## 6.1 Recommendations

The following recommendations are made from our study;

1. Pig farmers in Lusaka Province to adopt responsible antibiotic use through strict antimicrobial stewardship, enhance farm biosecurity, collaborate with veterinary authorities to monitor and control multidrug-resistant CoNS and implement proper hygiene practices as well as regular monitoring to reduce antimicrobial resistance and protect both animal and human health.
2. Research on Mechanisms of Resistance: The genetic mechanisms driving antibiotic resistance in MR-CoNS isolates require further investigation especially those that do not have the *mecA* gene. Alternative resistance determinants should be investigated using advanced molecular approaches.
3. Raising awareness and educating the public about the dangers of antimicrobial resistance (AMR) in livestock and its possible effects on human health by encouraging appropriate use of antibiotics and only use antibiotics when necessary.

4. Future studies should speciate all the isolates as well as look at all the resistance genes found in CoNS.

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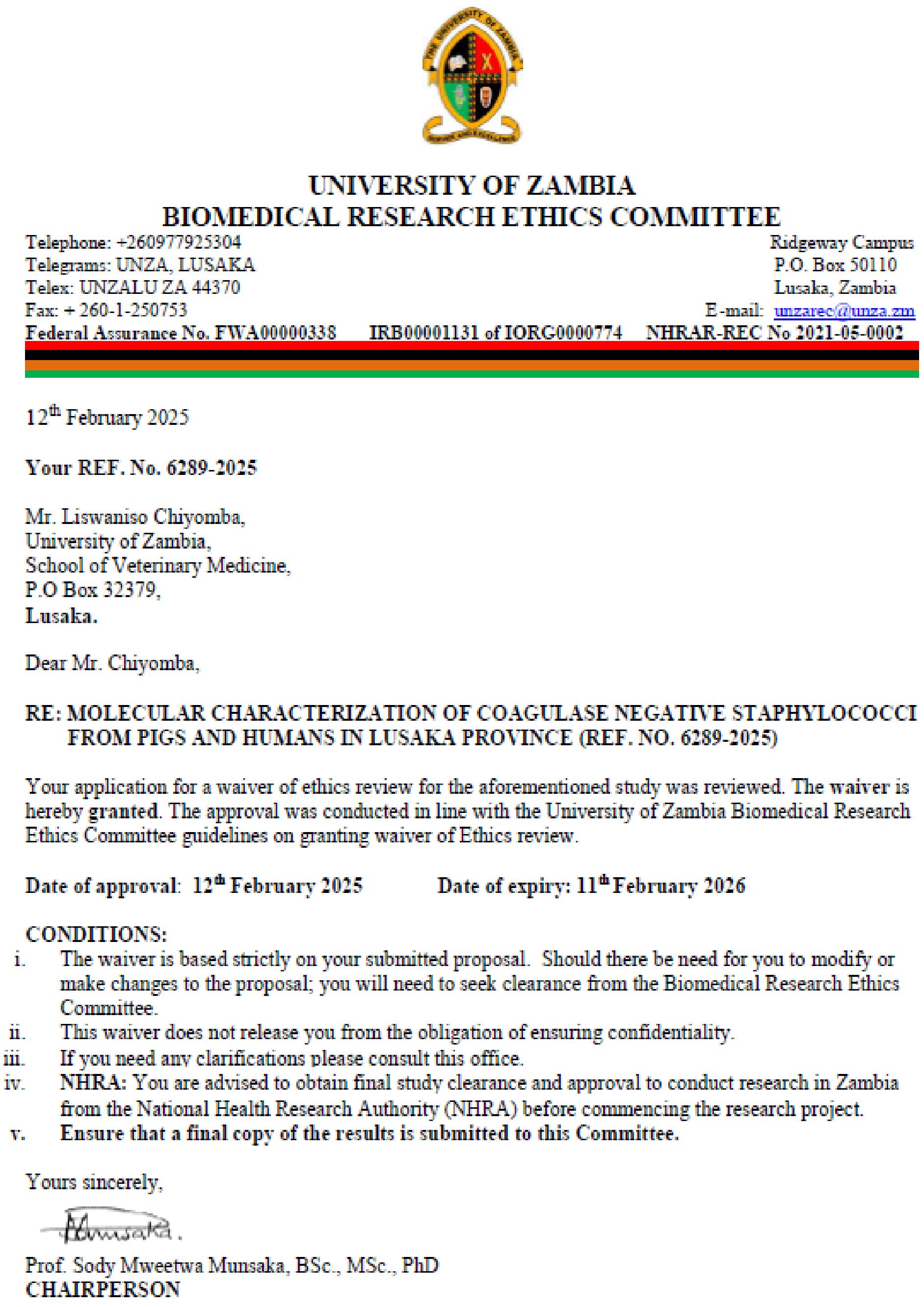
# Appendices

## Appendix A

## Ethical waver from UNZABREC



**UNIVERSITY OF ZAMBIA**
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**Federal Assurance No. FWA00000338 IRB00001131 of IORG0000774 NHRAR-REC No 2021-05-0002**

12th February 2025

**Your REF. No. 6289-2025**

Mr. Liswaniso Chiyomba,
University of Zambia,
School of Veterinary Medicine,
P.O Box 32379,
**Lusaka.**

Dear Mr. Chiyomba,

**RE: MOLECULAR CHARACTERIZATION OF COAGULASE NEGATIVE STAPHYLOCOCCI FROM PIGS AND HUMANS IN LUSAKA PROVINCE (REF. NO. 6289-2025)**

Your application for a waiver of ethics review for the aforementioned study was reviewed. The waiver is hereby **granted**. The approval was conducted in line with the University of Zambia Biomedical Research Ethics Committee guidelines on granting waiver of Ethics review.

**Date of approval: 12th February 2025** **Date of expiry: 11th February 2026**

**CONDITIONS:**

i. The waiver is based strictly on your submitted proposal. Should there be need for you to modify or make changes to the proposal; you will need to seek clearance from the Biomedical Research Ethics Committee.
ii. This waiver does not release you from the obligation of ensuring confidentiality.
iii. If you need any clarifications please consult this office.
iv. **NHRA:** You are advised to obtain final study clearance and approval to conduct research in Zambia from the National Health Research Authority (NHRA) before commencing the research project.
v. **Ensure that a final copy of the results is submitted to this Committee.**

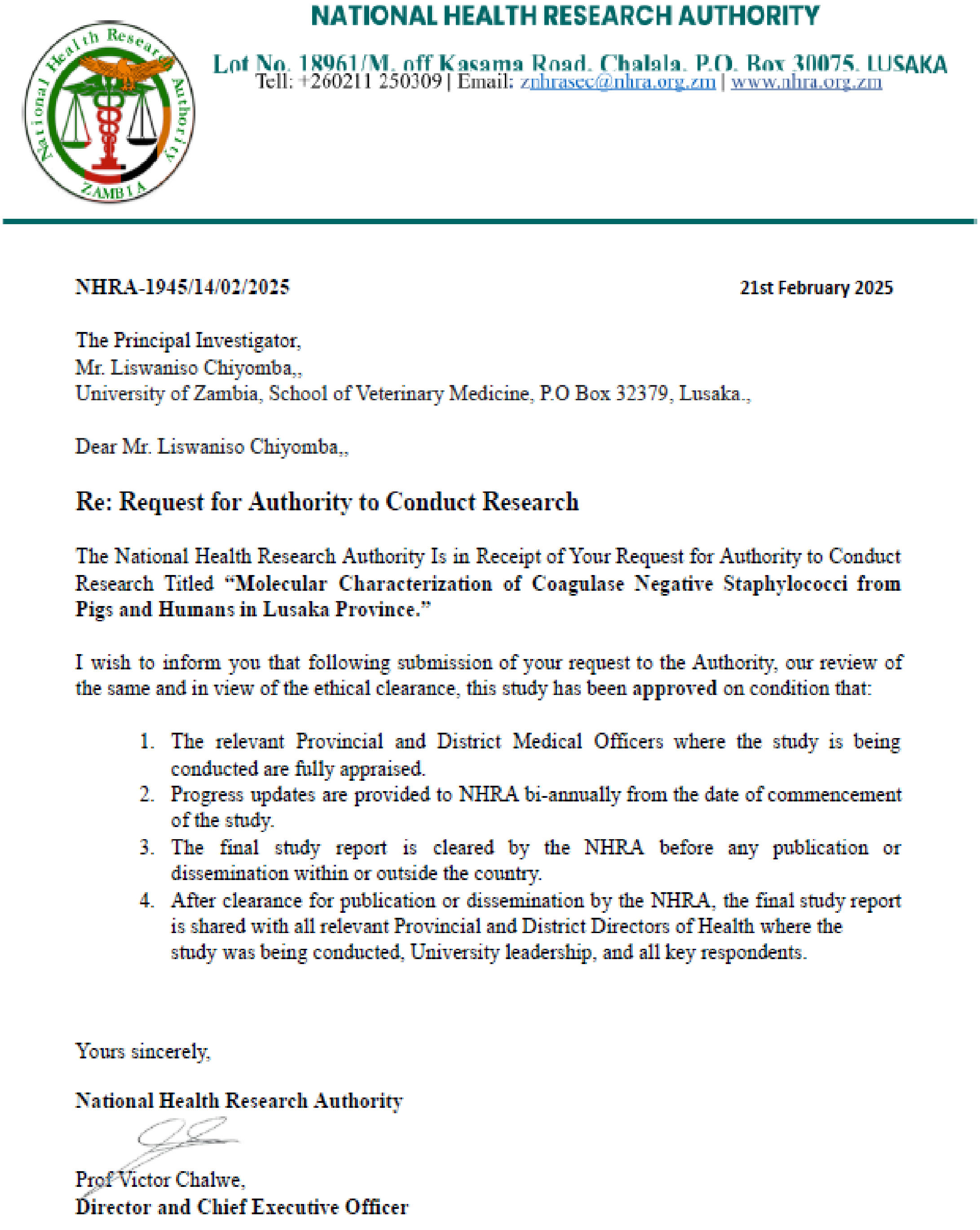
Yours sincerely,

Munsaka.

Prof. Sody Mweetwa Munsaka, BSc., MSc., PhD
CHAIRPERSON

## Appendix B

## Permission to Conduct Research from NHRA



NATIONAL HEALTH RESEARCH AUTHORITY
Lot No. 18961/M, off Kasama Road, Chalala, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@nhra.org.zm | www.nhra.org.zm

NHRA-1945/14/02/2025 21st February 2025

The Principal Investigator,
Mr. Liswaniso Chiyomba,,
University of Zambia, School of Veterinary Medicine, P.O Box 32379, Lusaka.,

Dear Mr. Liswaniso Chiyomba,,

**Re: Request for Authority to Conduct Research**

The National Health Research Authority Is in Receipt of Your Request for Authority to Conduct Research Titled **"Molecular Characterization of Coagulase Negative Staphylococci from Pigs and Humans in Lusaka Province."**

I wish to inform you that following submission of your request to the Authority, our review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised.
2. Progress updates are provided to NHRA bi-annually from the date of commencement of the study.
3. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country.
4. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, University leadership, and all key respondents.

Yours sincerely,

**National Health Research Authority**

Prof Victor Chalwe,
**Director and Chief Executive Officer**