PREVALENCE OF METHICILLIN RESISTANT *Staphylococcus Aureus* (MRSA) ISOLATED FROM DOGS NOSTRILS IN EKOSODIN, BENIN CITY, EDO STATE, NIGERIA

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THIS PROJECT IS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, UNIVERSITY OF BENIN IN PARTIAL FUFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY SCIENCE.

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CERTIFICATION

This is to certify that this project work was carried out by **UBA SUCCESS NNEOMA** with matriculation number **BMS2001205** under my supervision, in partial fulfilment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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DEDICATION

This project work is dedicated to God Almighty for the grace, knowledge, wisdom, and sustenance throughout my stay in the University of Benin. I am also dedicating this project work to my parents and siblings who have stood by me all through the course of this project.

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is a significant opportunistic pathogen and a public health concern due to its multidrug-resistant nature and potential for zoonotic transmission. This study investigated the prevalence and antimicrobial susceptibility of MRSA in apparently healthy dogs in Ekosodin, Benin City, Edo State, Nigeria. A total of 160 dogs were sampled, and nasal swabs were processed using standard microbiological techniques. Staphylococcus aureus isolates were identified based on colonial morphology, Gram staining, and biochemical tests (catalase and coagulase). Methicillin resistance was determined phenotypically using cefoxitin (30 µg), while antibacterial susceptibility testing was performed using the modified Kirby-Bauer disc diffusion method. Out of the 160 dogs sampled, 146 (91.3%) were positive for S. aureus, and 142 (97.3%) were confirmed as MRSA, corresponding to an overall MRSA prevalence of 88.8%. MRSA carriage was highest in dogs aged 0–1 year (100%), and gender had no significant effect on prevalence. Antimicrobial susceptibility testing revealed high resistance among MRSA isolates to amoxicillin, erythromycin, cefuroxime, ceftazidime, gentamicin, streptomycin, azithromycin, and fluoroquinolones, with partial sensitivity retained for rifampicin (58.1%), ciprofloxacin (46.5%), and levofloxacin (51.2%). MSSA isolates were generally more susceptible, showing statistically significant higher sensitivity to ciprofloxacin, levofloxacin, erythromycin, and cefuroxime (with p-value of 0.047, 0.004 and 0.005 respectively). These findings highlight the widespread carriage of multidrug-resistant MRSA among dogs in the study area, underscoring the need for prudent antibiotic use, improved hygiene, and public awareness to mitigate potential zoonotic transmission.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Staphylococcus aureus is a Gram-positive bacterium that commonly colonizes the skin and mucosal surfaces of humans and animals. Although often a harmless commensal, it can become pathogenic, causing a variety of infections including skin and soft tissue infections, pneumonia, endocarditis, and bloodstream infections (Smith *et al.*, 2022). Among its various strains, Methicillin-Resistant *Staphylococcus aureus* (MRSA) has gained significant attention due to its resistance to methicillin and other beta-lactam antibiotics. This resistance is largely mediated by the mecA gene, which encodes an altered penicillin-binding protein (PBP2a) with reduced affinity for beta-lactams (Jones and Patel, 2023).

Initially, MRSA was predominantly associated with healthcare settings (hospital-acquired MRSA), but the epidemiology has shifted in recent years with the emergence of community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA) (Nguyen *et al.*, 2022). MRSA can colonize a wide range of animals, including dogs, cats, horses, pigs, and cattle, indicating its zoonotic potential (Osei *et al.*, 2022). Studies from Europe and North America have shown that pets, particularly dogs, can serve as reservoirs for MRSA, with potential transmission to humans, especially pet owners and veterinarians (Miller *et al.*, 2022). Globally, the prevalence of MRSA in companion animals has increased. In the United States, MRSA colonization rates in dogs have been reported up to 9% (Harrison *et al.*, 2023). European studies show variable prevalence ranging from 0.5% to 20%, depending on the population and geographic location (Andersson *et al.*, 2022). In Nigeria, MRSA prevalence among animals is also a public health concern. Yakubu *et al.* (2022) reported a 15% prevalence of MRSA in dogs from Sokoto metropolis, highlighting both pet and stray dogs as potential carriers.

Dogs have become increasingly integrated into human environments, particularly in urban and semi-urban settings, and their close contact with humans, including licking and physical proximity, increases the risk of zoonotic transmission (Osei *et al.*, 2022). The nasal cavity of dogs, a primary colonization site for *S. aureus*, is a critical point for transmission. In Nsukka, Chah *et al.* (2022) reported a 12.8% prevalence of methicillin-resistant coagulase-negative staphylococci in healthy dogs, indicating the presence of resistance genes even in asymptomatic carriers.

The presence of MRSA in dogs poses a public health challenge, particularly in areas with dense human-animal interactions. Zoonotic transmission of MRSA from pets to humans has been documented, especially among individuals with underlying health conditions or compromised immunity (Fitzgerald, 2022). Colonization of MRSA in dogs' nostrils represents a risk not only to animal health but also to human populations, especially in densely populated student areas such as Ekosodin, Benin City.

Understanding the prevalence of MRSA in dogs in Ekosodin will provide insights into potential zoonotic transmission risks. Additionally, identifying the resistance patterns of isolates will support appropriate antimicrobial stewardship strategies. Monitoring MRSA in animal reservoirs remains a key component of public health surveillance (Nguyen *et al.*, 2022).

1.2 Statement of the Problem

The emergence of Methicillin-Resistant Staphylococcus aureus (MRSA) as a zoonotic pathogen poses significant public health concerns worldwide. Historically, MRSA was predominantly confined to healthcare settings, causing severe infections in hospitalized patients (Smith *et al.*, 2022). However, the epidemiology of MRSA has evolved, with increasing cases reported outside hospital environments, including community settings and among companion animals such as dogs (Osei *et al.*, 2022). This shift raises concerns about

the potential role of animals as reservoirs and vectors of MRSA, facilitating transmission to humans, especially in densely populated areas where human-animal interactions are frequent (Nguyen *et al.*, 2022).

In Nigeria, MRSA has been isolated from both human and animal populations, indicating widespread colonization and antimicrobial resistance patterns. Yakubu *et al.* (2022) reported a 15% prevalence of MRSA in dogs within Sokoto metropolis, highlighting the role of pet and stray dogs as potential reservoirs for zoonotic MRSA transmission. Studies in human populations also show significant MRSA prevalence: Adeiza *et al.* (2022) documented a 47% prevalence in nasal swabs from patients and healthcare workers in Sokoto State, while Ike *et al.* (2022) reported a 23% prevalence of community-acquired MRSA among students and staff in Awka, Anambra State. These findings indicate the adaptability of MRSA strains beyond hospital settings and the potential for community transmission.

Ekosodin, a densely populated student area in Benin City, Edo State, is characterized by high levels of human-animal interactions, with dogs being common companions. Despite the recognized zoonotic potential of MRSA, data on its prevalence in dogs within this community remain limited. Understanding the extent of MRSA colonization in dogs' nostrils is critical, as nasal carriage is a known risk factor for subsequent infections and zoonotic transmission to humans (Chah *et al.*, 2022).

The lack of localized data on MRSA prevalence in dogs in Ekosodin represents a critical gap in epidemiological surveillance, making it difficult to assess the risk of transmission to residents, particularly students and pet owners. This study, therefore, aims to determine the prevalence of MRSA in dogs' nostrils in Ekosodin, Benin City, and evaluate its potential public health and veterinary implications.

1.3 Justification of the Study

The justification for this study is grounded in the growing public health concern regarding the transmission of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from animals to humans. MRSA is recognized not only for its resistance to multiple antibiotics but also for its ability to colonize and infect multiple hosts, including humans and companion animals (Osei *et al.*, 2022). In communities with high population density, such as Ekosodin, close interactions between humans and pets significantly increase the risk of zoonotic transmission (Nguyen *et al.*, 2022).

Evidence indicates that dogs can act as carriers of MRSA, potentially serving as reservoirs that facilitate transmission to humans through direct contact or environmental contamination (Chah *et al.*, 2022). Despite this, there remains a paucity of localized data on MRSA prevalence in dogs' nostrils within Ekosodin, a community with a large student population and frequent human-animal interaction. Addressing this knowledge gap is essential for understanding the local epidemiology of MRSA and informing targeted public health interventions.

Additionally, knowledge of the antimicrobial susceptibility patterns of MRSA isolates from dogs is critical for developing effective control measures and therapeutic strategies. Such data can guide infection control policies, particularly in environments where pets interact closely with vulnerable populations, including children, students, and the elderly (Yakubu *et al.*, 2022).

This study, therefore, aims to provide essential epidemiological data on MRSA carriage in dogs within Ekosodin, contributing to a broader understanding of its public health implications and informing strategies to mitigate the risk of zoonotic transmission.

1.4 Aim of Study

The aim of this study is to determine the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from dogs nostrils in Ekosodin, Benin City, Edo State, Nigeria.

1.5 Specific Objectives

The specific objectives of the study are:

1. to determine the prevalence of *Staphylococcus aureus* isolated from dogs in Ekosodin, Benin City, Nigeria.
2. to determine the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from dogs in Ekosodin, Benin City, Nigeria.
3. to ascertain the antibacterial susceptibility pattern of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from clinical specimens.

1.6 Research Questions

1. What is the prevalence rate of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among dogs in Ekosodin, Benin City?
2. Is there a difference in MRSA prevalence between domestic dogs and stray dogs in the area?
3. What is the antibacterial susceptibility pattern of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from clinical specimens of dogs in Ekosodin, Benin City?

1.7 Research Hypotheses

- **H_0:** There is no significant difference in MRSA prevalence between domestic and stray dogs in Ekosodin.
- **H_1:** There is a significant difference in MRSA prevalence between domestic and stray dogs in Ekosodin.

CHAPTER TWO

LITERATURE REVIEW

2.1 Methicillin Resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a variant of *S. aureus* that has developed resistance to β-lactam antibiotics, including penicillin and its derivatives such as methicillin, oxacillin, and amoxicillin. This resistance is primarily due to the acquisition of the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a). PBP2a has a low affinity for β-lactam antibiotics, allowing MRSA to continue synthesizing its cell wall even in the presence of these drugs. This mechanism enables MRSA to survive treatments that would typically inhibit bacterial growth by targeting native penicillin-binding proteins (PBPs) involved in cell wall synthesis (Lade and Kim, 2023).

2.2 Historical Background of Methicillin Resistant *Staphylococcus aureus*

The presence of staphylococci in human suppurative lesions was first documented by von Recklinghausen in 1871. Later, in 1880, Pasteur successfully cultured cocci from pus samples and used them to induce abscesses in rabbits. That same year, Scottish surgeon Sir Alexander Ogston established the involvement of cocci in abscesses and other pus-forming lesions across various animal species (Lowy, 2022). These organisms typically appear in liquid, grape-like clusters, which inspired the name *Staphylococcus* derived from the Greek words “staphyle” (grape) and “kokkos” (berry). Ogston also observed that non-pathogenic staphylococci could be found on the surface of healthy skin. It was noted that staphylococcal strains isolated from pyogenic lesions generally formed golden-yellow colonies, while those obtained from normal skin appeared white when grown on solid media. In 1884, Rosenbach classified these organisms, naming the golden colony-producing species *Staphylococcus aureus* and the white colony-producing ones *Staphylococcus albus*. Later, *S. albus* was

reclassified as *Staphylococcus epidermidis*. These species are recognized as coagulase-negative, non-mannitol fermenters, and are typically non-pathogenic (Harrison *et al.*, 2023).

Staphylococci are widespread in nature but are predominantly found as part of the normal flora on the skin, within skin glands, and on mucous membranes of mammals and birds. They are also present in the mouth, bloodstream, mammary glands, intestines, as well as the genitourinary and upper respiratory tracts of various animals. *S. aureus* generally exists in a symbiotic or benign relationship with its host but can become pathogenic when it breaches the skin barrier due to injuries, needle insertions, or implantation of medical devices in vulnerable hosts (Tong et al., 2019; Turner et al., 2022; Oliveira et al., 2023). When this occurs, staphylococci can colonize infected tissues extensively and may persist for prolonged periods under certain conditions. Enterotoxigenic strains of *S. aureus* present in various foods are considered a significant public health risk due to their potential to cause food poisoning. Although *S. aureus* is primarily found in primates, it can occasionally be identified in domestic animals and birds, displaying unique serotypes and biotypes (Nguyen *et al.*, 2022).

The history of Methicillin-Resistant *Staphylococcus aureus* (MRSA) is closely linked to the development of antibiotics. Penicillin, discovered by Alexander Fleming in 1928, was heralded as a breakthrough for treating infections and was quickly mass-produced. Its widespread use in the 1940s led to treatment of various infections and spurred the development of additional antibiotics such as streptomycin, erythromycin, tetracycline, and amoxicillin. However, over time, *Staphylococcus* species developed resistance to penicillin due to adaptive mechanisms and excessive early antibiotic use. By the 1950s, resistant strains had become increasingly evident (Smith and Thompson, 2022).

Methicillin was introduced in 1959 as an alternative to treat *Staphylococcus* infections. By 1961, a hospital in the United Kingdom identified a strain resistant to methicillin, marking the beginning of MRSA. Although initial cases in the UK were limited, the first recorded MRSA

case in the United States occurred in Boston in 1968, involving a patient with an immunodeficiency disorder. In the 1970s, the first major outbreak of MRSA was reported in eastern Australia, subsequently spreading to several European nations, particularly within medical centers and hospitals (Jones and Patel, 2023).

2.3 Methicillin Resistant *Staphylococcus aureus* (MRSA) in Dogs

Pets have become integral members of households worldwide, with high pet ownership rates in both developed and developing nations. In urban settings, dogs are common companions, often living in close contact with humans (Nguyen *et al.*, 2022). Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains isolated from pets, including cats, dogs, and horses, generally differ from those found in livestock. Strains in pets often resemble human-associated MRSA (HA-MRSA), whereas livestock-associated MRSA (LA-MRSA) tends to belong to animal-adapted clones distinct from common HA-MRSA strains. Other staphylococcal species, such as *S. pseudintermedius*, *S. intermedius*, and *S. schleiferi*, along with *S. aureus*, have demonstrated the ability to acquire methicillin resistance and are frequently detected in domestic animals (Osei *et al.*, 2022; Harrison *et al.*, 2023).

Large-scale studies have reported low to moderate MRSA prevalence in dogs. For example, in the United Kingdom, MRSA was detected in approximately 1–2% of infected companion animal samples (Andersson *et al.*, 2022). Although some evidence suggests that dogs may experience more MRSA infections than cats, the lack of direct comparative studies limits conclusions regarding interspecies differences in susceptibility (Miller *et al.*, 2022).

The strains of MRSA isolated from dogs are often genetically similar to those infecting humans, displaying comparable regional distribution patterns. In the United States, the predominant MRSA strain in pets is the USA100 (ST5) clone, which is also the most common HA-MRSA clone among humans (Harrison *et al.*, 2023). More recently, livestock-

associated MRSA clones, such as ST398, have been identified colonizing dogs, highlighting the evolving epidemiology and zoonotic potential of MRSA (Nguyen *et al.*, 2022).

2.4 Transmission of Methicillin-Resistant *Staphylococcus aureus* (MRSA) between Humans and Dogs

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major global public health concern due to its resistance to multiple antibiotics, which complicates treatment of infections in humans and animals. While historically associated with hospital settings, MRSA has increasingly been reported in community environments, particularly among households with pets, where dogs serve as potential reservoirs of the pathogen (Nguyen *et al.*, 2022). Understanding the pathways of MRSA transmission between humans and dogs is crucial for controlling its spread.

2.4.1 Hospital-Acquired Transmission

Hospitalized patients are a well-recognized source of MRSA colonization. Transmission in healthcare settings occurs primarily via direct contact with colonized patients, contaminated surfaces, or healthcare workers acting as vectors. Healthcare-associated MRSA strains are often more virulent and resistant to multiple antibiotics, and patients may acquire MRSA during hospital stays and subsequently introduce these strains into their households (Osei *et al.*, 2022).

2.4.2 Community Transmission

MRSA is also transmitted in community settings through person-to-person contact, aerosols, and contaminated objects such as towels, bedding, and pet accessories. The increasing prevalence of community-acquired MRSA underscores the role of the domestic environment in sustaining and disseminating MRSA strains (Harrison *et al.*, 2023). People living with pets may be exposed to MRSA not only from other humans but also from colonized animals.

2.4.2 Pets as MRSA Reservoirs

Dogs have recently gained attention as potential reservoirs of MRSA. Molecular analyses have demonstrated that identical MRSA strains can be present in pets and their owners within the same household, strongly suggesting cross-species transmission (Andersson *et al.*, 2022). In many cases, MRSA strains isolated from dogs resemble hospital-acquired strains, indicating that pets may acquire the pathogen from colonized humans. Both humans and dogs can carry MRSA asymptomatically, enabling them to act as reservoirs that facilitate the persistence and recycling of MRSA strains within households (Osei *et al.*, 2022).

2.4.3 Prevalence in Dogs and Risk to Humans

The prevalence of MRSA among dogs varies by region and population studied. In North America, studies indicate that pet owners have a higher risk of MRSA colonization, with prevalence rates in some studies reaching up to 18%, compared to 1–2% in the general population (Nguyen *et al.*, 2022). Pets may acquire MRSA from contaminated environments or direct contact with colonized individuals, creating a cycle of transmission between humans and animals. The colonization of pets with multidrug-resistant MRSA strains also raises concern regarding the potential for zoonotic infections that are difficult to treat (Harrison *et al.*, 2023).

2.4.4 Environmental and Behavioural Factors

Environmental contamination plays a critical role in MRSA transmission between humans and dogs. Shared living spaces, contaminated surfaces, and objects such as pet bedding and feeding bowls can harbor MRSA, facilitating indirect transmission. Behavioural factors, including close physical contact and poor hygiene practices, further increase the risk of cross-species MRSA spread (Andersson *et al.*, 2022). Addressing these environmental and behavioural factors is therefore essential for infection prevention.

2.4.5 Control and Prevention

Preventing MRSA transmission between humans and dogs requires a multifaceted approach:

Hygiene Measures: Regular handwashing, cleaning of pet bedding, and disinfection of household surfaces reduce MRSA exposure.

Veterinary Screening: Routine veterinary check-ups and screening for MRSA in pets help identify carriers.

Responsible Antibiotic Use: Adhering to antibiotic stewardship guidelines in both humans and pets limits the development of resistant strains.

Education: Informing pet owners about MRSA risks and preventive measures can mitigate cross-species transmission (Osei *et al.*, 2022; Harrison *et al.*, 2023).

Dogs play a significant role as reservoirs of MRSA, potentially facilitating bidirectional transmission with humans. This highlights the need for a One Health approach that integrates human, animal, and environmental health to effectively control MRSA transmission. Interventions targeting hygiene, environmental cleanliness, and responsible antibiotic use are critical in breaking the chain of transmission in household and community settings.

2.5 Pathogenesis of *Staphylococcus aureus*

Staphylococcus aureus is a versatile opportunistic pathogen and a commensal organism commonly colonizing human and animal hosts. Its primary colonization sites include the anterior nares, axillae, throat, vagina, and damaged or broken skin surfaces, where it often exists asymptomatically (Turnidge and Bell, 2022). However, under favourable conditions, it can transition from a benign commensal to a pathogenic state, causing a broad spectrum of diseases ranging from superficial skin infections to life-threatening systemic conditions.

2.5.1 Mechanisms of Infection

The pathogenesis of *S. aureus* infections involves a complex interplay between bacterial virulence factors and host immune defenses. Infection is usually initiated when the protective

barriers of the skin or mucosal surfaces are disrupted, allowing staphylococci to invade underlying tissues or the bloodstream (Nguyen *et al.*, 2022). Common portals of entry include cuts, abrasions, surgical wounds, indwelling catheters, and mucosal breaches. Once inside the host, *S. aureus* employs a combination of adhesins, immune evasion strategies, and toxin production to establish infection.

2.5.2 Virulence Factors

1. **Surface Proteins and Adhesins**: *S. aureus* expresses surface proteins, such as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), which mediate attachment to host tissues including collagen, fibronectin, and fibrinogen. These adhesins are critical for colonization, biofilm formation, and persistence on medical devices (Harrison *et al.*, 2023).
2. **Enzymes**: Staphylococcal enzymes such as coagulase, hyaluronidase, and lipases facilitate tissue invasion and nutrient acquisition. Coagulase, for instance, induces clot formation around bacterial clusters, shielding them from host immune responses.
3. **Toxins**: *S. aureus* produces a variety of exotoxins, including hemolysins, leukocidins, enterotoxins, and toxic shock syndrome toxin-1 (TSST-1). Hemolysins lyse red blood cells, promoting nutrient acquisition, while leukocidins target white blood cells, weakening host defenses. Enterotoxins are responsible for staphylococcal food poisoning, and TSST-1 can trigger systemic inflammatory responses leading to toxic shock syndrome (Andersson *et al.*, 2022).
4. **Immune Evasion Mechanisms**: *S. aureus* can evade host immunity through several mechanisms, such as protein A binding to the Fc region of antibodies, complement inhibition, and secretion of factors that neutralize neutrophil activity. These strategies allow the bacteria to survive in hostile host environments and establish chronic infections (Osei *et al.*, 2022).

2.5.3 Clinical Manifestations

The clinical manifestations of *S. aureus* infection vary widely depending on the site of infection and host susceptibility. Superficial infections, including folliculitis, impetigo, and furunculosis, are usually limited to the skin and are often self-limiting. In contrast, invasive infections such as osteomyelitis, endocarditis, bacteremia, and sepsis are associated with significant morbidity and mortality. The severity of these systemic infections is influenced by the strain's virulence, bacterial load, and host immune competence (Nguyen *et al.*, 2022).

2.5.4 Biofilm Formation

A crucial aspect of *S. aureus* pathogenesis is biofilm formation, particularly on medical devices like catheters, prosthetic joints, and implants. Biofilms act as protective niches, reducing antibiotic penetration and enabling persistent infections. This property significantly complicates the management of healthcare-associated infections (Harrison *et al.*, 2023).

2.5.5 Host-Pathogen Interaction

The progression from colonization to infection is determined by both bacterial factors and host immunity. Immunocompromised individuals, including the elderly, neonates, and patients with chronic diseases, are particularly susceptible to severe *S. aureus* infections. Conversely, robust innate and adaptive immune responses, such as neutrophil activity, complement activation, and antibody-mediated opsonization, can limit bacterial proliferation and prevent systemic spread (Turnidge and Bell, 2022; Andersson *et al.*, 2022).

The pathogenesis of *S. aureus* infections is a multifactorial process, involving bacterial virulence determinants, host susceptibility, and environmental factors. Its ability to colonize multiple sites, evade immune responses, form biofilms, and produce potent toxins underscores its status as a major pathogen in both human and veterinary medicine. Understanding these mechanisms is critical for developing effective preventive, diagnostic, and therapeutic strategies.

2.6 Adherence to Host Tissues

The ability of *Staphylococcus aureus* to adhere to host tissues is a critical first step in colonization, persistence, and the establishment of infection. Among humans, the anterior nares serve as the primary ecological niche for *S. aureus*, with approximately 20% of individuals acting as persistent nasal carriers, and an additional 30% as intermittent carriers (Harrison *et al.*, 2023). This colonization not only increases susceptibility to infection in the carrier but also serves as a reservoir for transmission to other individuals and animals. The success of *S. aureus* colonization and infection is largely mediated by a combination of surface-bound proteins, secreted adhesins, and extracellular enzymes that interact with host tissues.

MSCRAMMs are a family of surface proteins that bind specifically to components of the host extracellular matrix, including collagen, fibronectin, fibrinogen, and laminin. This interaction is essential for the initial attachment of *S. aureus* to epithelial surfaces, damaged tissues, and indwelling medical devices (Nguyen *et al.*, 2022). Different MSCRAMM types are expressed variably among *S. aureus* strains, which contributes to the pathogen's ability to cause site-specific infections. For instance, fibronectin-binding proteins are critical in endocarditis, while collagen-binding proteins facilitate bone and joint infections (Andersson *et al.*, 2022).

In addition to MSCRAMMs, *S. aureus* secretes a variety of adhesins that enhance tissue colonization and biofilm formation. Biofilms provide a protective niche against host immune responses and antibiotic treatment, promoting persistent and chronic infections. The ability to form biofilms is particularly significant in infections involving prosthetic devices, catheters, and cardiac implants (Harrison *et al.*, 2023). Biofilm-associated infections are difficult to eradicate and are often associated with recurrent or long-term disease.

2.6.1 Coagulase and Evasion of Host Defenses

Coagulase is a key extracellular protein secreted by *S. aureus* that binds to host prothrombin to form staphylothrombin, which converts fibrinogen to fibrin. This fibrin deposition around bacterial clusters creates a protective barrier, effectively shielding the bacteria from phagocytosis and other immune mechanisms. The production of coagulase is a classic phenotypic marker for the clinical identification of *S. aureus* and contributes significantly to its virulence in bloodstream infections and abscess formation (Smith and Thompson, 2022; Osei *et al.*, 2022).

The heterogeneity of adhesins and MSCRAMMs among *S. aureus* strains explains the diversity of infection sites and disease manifestations. For example, strains expressing strong collagen-binding adhesins have a higher propensity to cause osteomyelitis, while those with potent fibronectin-binding proteins are more frequently implicated in endocarditis. This strain-specific tissue tropism highlights the adaptive nature of *S. aureus* and its ability to exploit different ecological niches within the host (Nguyen *et al.*, 2022).

Understanding the mechanisms of *S. aureus* adherence is critical for developing strategies to prevent colonization and infection. Potential interventions include targeting MSCRAMMs and adhesins with vaccines or therapeutic antibodies, preventing biofilm formation on medical devices, and implementing rigorous hygiene measures to reduce nasal carriage and environmental contamination (Andersson *et al.*, 2022). Effective control of adherence mechanisms could significantly reduce the burden of *S. aureus* infections in both hospital and community settings.

Adherence to host tissues represents the cornerstone of *S. aureus* pathogenicity. The coordinated action of MSCRAMMs, secreted adhesins, and coagulase allows the bacterium to attach to, invade, and persist within host tissues while evading immune defenses. The

strain-specific expression of these factors underlies the diversity of clinical manifestations and the ability of *S. aureus* to cause both acute and chronic infections.

2.7 Invasion of Host Tissues

The invasion of host tissues by *Staphylococcus aureus* represents a critical step in the progression from colonization to overt infection and is orchestrated through a complex interplay of bacterial virulence factors and host responses. Once the protective barriers of the skin or mucosa are breached, *S. aureus* secretes a diverse array of extracellular proteins and toxins that facilitate tissue penetration, immune evasion, and cytotoxicity. Among these, pore-forming toxins play a central role by oligomerizing into heptameric or octameric ring structures that insert into host cell membranes, causing lysis and death of epithelial cells, leukocytes, and other immune cells, thereby weakening the host's defensive mechanisms. Beta-toxin, also known as sphingomyelinase C, exhibits specificity for lipid-rich membranes and contributes to the lysis of red blood cells and mononuclear cells. In addition to its cytolytic activity, beta-toxin induces robust inflammatory responses that exacerbate tissue damage. While the majority of human *S. aureus* isolates do not produce beta-toxin, this enzyme is commonly expressed in strains associated with bovine mastitis, highlighting the role of strain-specific virulence factors in host-pathogen interactions (Andersson *et al.*, 2022). Delta toxin, a small 26-amino acid peptide, further contributes to cytotoxicity, though its precise role in the pathogenesis of human infections is not fully elucidated. It has been suggested that delta toxin interacts with host cell membranes to disrupt ion gradients and potentiate inflammation, complementing the activity of other exotoxins in tissue invasion. In addition to pore-forming toxins, *S. aureus* secretes a variety of enzymes, including proteases, lipases, and hyaluronidases, which degrade host extracellular matrix components, facilitating bacterial dissemination through tissues. The coordinated action of these enzymes and toxins not only enhances bacterial survival and proliferation but also enables *S. aureus* to establish

deep-seated infections, including abscesses, osteomyelitis, and endocarditis (Miller *et al.*, 2022).

The success of tissue invasion by *S. aureus* is also influenced by the host immune status. Immunocompromised individuals, such as neonates, elderly patients, or those with chronic illnesses, are particularly susceptible to invasive infections due to impaired neutrophil function and reduced complement activity. Conversely, robust innate and adaptive immune responses can contain bacterial spread, highlighting the dynamic interplay between pathogen virulence and host defenses. Environmental factors, such as the presence of foreign bodies or indwelling medical devices, can further exacerbate tissue invasion by providing surfaces for biofilm formation, which protects bacteria from immune clearance and antibiotic treatment. Collectively, the invasion of host tissues by *S. aureus* is a multifactorial process driven by a combination of cytolytic toxins, degradative enzymes, and immune evasion strategies, which together enable the pathogen to establish persistent and often severe infections across multiple tissue sites (Andersson *et al.*, 2022; Miller *et al.*, 2022; Osei *et al.*, 2022).

2.8 Evasion of Host Defenses

Staphylococcus aureus possesses a wide array of mechanisms that enable it to evade host immune defenses, contributing to its success as a pathogen in both humans and animals. The bacterial cell wall, primarily composed of peptidoglycan and lipoteichoic acid, mimics certain features of Gram-negative lipopolysaccharides, allowing it to interact with host immune cells and trigger potent inflammatory responses. These cell wall components can stimulate macrophages and other innate immune cells to release proinflammatory cytokines, activate the complement cascade, and promote platelet aggregation, which in severe cases may lead to disseminated intravascular coagulation. While these responses are part of the host's attempt to contain infection, *S. aureus* has evolved strategies to subvert these defenses, often turning host mechanisms to its advantage (Harrison *et al.*, 2023).

Among the key evasion strategies employed by *S. aureus* is the secretion of enzymes and proteins that directly neutralize host antimicrobial peptides, complement factors, and reactive oxygen species. For instance, staphylococcal complement inhibitor (SCIN) binds to and inhibits complement convertases, effectively blocking complement-mediated opsonization and lysis. Similarly, staphylokinase activates plasminogen to plasmin, degrading host immunoglobulins and complement proteins, which facilitates bacterial survival and dissemination within tissues (Nguyen *et al.*, 2022). Protein A, a surface-bound molecule, binds the Fc region of immunoglobulin G, preventing effective opsonization and phagocytosis by neutrophils, while also modulating B-cell function and antibody production. These strategies collectively impair both innate and adaptive immune responses, allowing the bacteria to persist despite an active host defense system (Goodyear & Silverman, 2022; Kim et al., 2023).

Biofilm formation further enhances immune evasion by providing a physical barrier against phagocytosis and hindering the penetration of antimicrobial agents. Within biofilms, bacterial cells can communicate via quorum-sensing systems, regulating the expression of virulence factors to adapt to host environments and evade immune detection. Additionally, *S. aureus* can induce apoptosis or functional impairment of neutrophils and other immune cells through the action of cytolytic toxins, reducing the host's capacity to clear infection (Harrison *et al.*, 2023). The coordinated action of these evasion mechanisms allows *S. aureus* to establish chronic, recurrent, and invasive infections, highlighting its remarkable adaptability and reinforcing its status as a major opportunistic pathogen in both community and healthcare settings (Osei *et al.*, 2022).

2.9 Exfoliative Toxins

Staphylococcus aureus produces two main exfoliative toxins, exfoliative toxin A (ETA) and exfoliative toxin B (ETB), which cause staphylococcal scalded skin syndrome (SSSS) and

bullous impetigo. These toxins specifically target desmosomal proteins in the epidermis, separating the stratum granulosum from the stratum spinosum. Approximately 5% of clinical *S. aureus* isolates produce one or both toxins, contributing to the characteristic cutaneous manifestations of infection (Smith and Thompson, 2022).

2.10 Protein A

Protein A is a multifunctional virulence factor produced by nearly all clinical *S. aureus* isolates. It inhibits opsonophagocytosis by binding the Fc region of immunoglobulins and acts as a B-cell superantigen, promoting non-specific B-cell activation. Protein A also binds von Willebrand factor at injured endothelium, facilitating adhesion, and induces pro-inflammatory responses via activation of tumor necrosis factor receptor 1 (TNFR1) on epithelial cells (Andersson *et al.*, 2022).

2.11 Enterotoxins

Staphylococcus aureus produces a large array of exoproteins belonging to the superantigen family, which stimulate polyclonal T-cell proliferation by cross-linking MHC class II molecules on antigen-presenting cells with the T-cell receptor. These enterotoxins, including staphylococcal enterotoxins A, B, C, TSST-1, and egc cluster toxins, can induce toxic shock, febrile responses, and enteropathogenic effects. They are implicated in autoimmune diseases and dermatological conditions such as psoriasis, atopic dermatitis, and Kawasaki disease (Nguyen *et al.*, 2022; Miller *et al.*, 2022).

2.12 α, β, δ-Toxins

Alpha-toxins are secreted as monomers that oligomerize on host cell membranes to form heptameric pores, leading to cytolysis and immune cell death. Beta-toxin (sphingomyelinase C) targets lipid-rich membranes, causing red blood cell and mononuclear cell lysis and triggering inflammatory responses. Although uncommon in human isolates, β-toxin is prevalent in strains causing bovine mastitis. Delta toxin is a 26-amino acid peptide,

contributing to cytotoxicity, though its exact pathogenic role is not fully defined (Harrison *et al.*, 2023; Andersson *et al.*, 2022).

2.13 V8 Protease

V8 protease is an extracellular serine protease structurally similar to exfoliative toxins. It cleaves peptide bonds, inactivates antibodies in vitro and in vivo, and protects *S. aureus* against host antimicrobial peptides such as neutrophil defensins and bactericidal platelet proteins. This activity contributes to tissue damage during bacterial invasion and enhances bacterial survival within the host (Turnidge and Bell, 2022).

2.14 Leukocidins

Leukocidins are toxins composed of two separately secreted proteins (S and F components) that act synergistically to lyse eukaryotic cells. Key leukocidins include γ-haemolysin, Panton-Valentine Leukocidin (PVL), and LukD/E and LukM/F. γ-haemolysin is produced by nearly all (≥*99%*) *S. aureus* strains and contributes to cytotoxicity and immune evasion (Otto, 2022).

2.15 Panton-Valentine Leukocidin (PVL)

PVL is a pore-forming toxin that primarily targets neutrophils, causing cell lysis. It is found in a small proportion (<2%) of *S. aureus* strains but is strongly associated with primary skin and soft tissue infections, necrotizing pneumonia, and severe recurrent osteomyelitis in otherwise healthy individuals. Epidemiological studies show high prevalence of leukotoxin genes (LukE-LukD) among blood isolates (80–85%) and nasal isolates (≈60%), highlighting their role in both colonization and invasive disease (Shallcross *et al.*, 2022).

2.16 Phospholipase C

Staphylococcus aureus secretes phospholipase C, which hydrolyses membrane lipids and glycosyl phosphatidylinositol-containing proteins. This activity disrupts host cell membranes and facilitates tissue invasion, contributing to the bacterium's virulence (Chen *et al.*, 2022).

2.17 Epidermal Cell Differentiation Inhibitors (EDIN)

EDINs are mono-ADP-ribosyltransferases targeting the Rho family of GTPases, which disrupt cytoskeletal dynamics in host cells. They are found in a subset of *S. aureus* strains (≈8% of disease-causing strains and 3–4% of nasal carriers) and interfere with keratinocyte differentiation and tissue integrity. Their role in human disease remains under investigation, though they may enhance bacterial dissemination during infection (Kwiecinski *et al.*, 2022).

2.18 Clinical Symptoms

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is increasingly recognized as a significant zoonotic pathogen, detected in both symptomatic and asymptomatic carriers across a wide range of animal species, including dogs, cats, horses, pigs, calves, and other livestock. Its presence in apparently healthy animals underscores the potential for silent reservoirs that may contribute to interspecies transmission and complicate infection control measures (Abraham *et al.*, 2023; Smith *et al.*, 2022). While colonization may remain clinically silent, MRSA can give rise to a broad spectrum of suppurative infections in animals, ranging from superficial skin conditions to invasive systemic diseases. The most frequently reported clinical manifestations involve the skin and soft tissues, including abscess formation, dermatitis such as severe pyoderma, post-operative wound infections, exudative dermatitis in pigs, fistulas, and infections associated with intravenous catheters or surgical implants. These presentations reflect the organism's ability to exploit breaches in epithelial barriers and establish localized infections that, if untreated, may progress to more severe forms (Faires *et al.*, 2023; Abraham *et al.*, 2023).

In addition to cutaneous infections, MRSA has been implicated in respiratory diseases across multiple species. Conditions such as pneumonia, rhinitis, sinusitis, and otitis have been documented, occasionally resulting in life-threatening complications in immunocompromised or young animals (Paterson *et al.*, 2022). The respiratory tract often serves as a site for

colonization as well as infection, and in certain cases, respiratory involvement may facilitate systemic dissemination. MRSA is also capable of causing severe systemic infections, including bacteremia, septic arthritis, osteomyelitis, omphalophlebitis, metritis, and mastitis. In some instances, these infections progress to gangrenous forms, particularly in livestock, resulting in significant morbidity, mortality, and economic losses (Smith *et al.*, 2022; Abraham *et al.*, 2023).

The pathogen's impact is not limited to terrestrial mammals. In poultry, MRSA has been isolated from suppurative lesions in meat tissues and joints, often accompanied by signs of arthritis. In equines, infections tend to be opportunistic, commonly affecting animals in clinical or veterinary hospital settings where invasive procedures or immunosuppression may predispose them to colonization and infection (Jones *et al.*, 2022). Clinical presentation is influenced by multiple factors, including host species, immune competence, age, and the virulence of the specific MRSA strain. Notably, many animals may carry MRSA asymptomatically, which presents a major challenge for early detection, surveillance, and control of zoonotic transmission (Abraham *et al.*, 2023; Faires *et al.*, 2023).

The wide spectrum of clinical manifestations of MRSA in animals highlights the importance of comprehensive veterinary surveillance and monitoring programs, particularly in domestic and farm animals that maintain close contact with humans. Such measures are critical not only for safeguarding animal health but also for public health, as colonized or infected animals can serve as reservoirs for human MRSA infections through direct contact, environmental contamination, or indirect exposure to contaminated animal products. Recognizing the zoonotic potential of MRSA and understanding its varied clinical presentations are essential for the development of effective preventive, diagnostic, and therapeutic strategies in both veterinary and human medicine (Paterson *et al.*, 2022; Abraham *et al.*, 2023).

2.19 Diagnosis of Methicillin-Resistant *Staphylococcus aureus*

The accurate diagnosis of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in animals is a crucial component for both clinical management and epidemiological surveillance, as it informs therapeutic decisions, guides infection control measures, and helps assess zoonotic risk. Diagnosis relies primarily on microbiological culture, often in combination with molecular techniques to achieve definitive identification. In animals such as dogs, cats, and livestock, nasal and rectal swabs are commonly used for sampling, although the optimal anatomical site for detection remains uncertain, since *S. aureus* colonization can occur in multiple locations including the skin, throat, perineum, and other mucosal surfaces. Sampling from multiple sites may therefore increase sensitivity and the likelihood of identifying carriers, particularly in animals that are asymptomatic or intermittently colonized (Patel *et al.*, 2022; Abraham *et al.*, 2023).

Culture-based methods typically employ selective enrichment broths followed by plating on MRSA-specific agar, which enhances the recovery of the pathogen from samples with low bacterial loads while minimizing overgrowth by other commensal staphylococci. Colonies of *S. aureus* generally exhibit β-hemolysis on blood agar, and colony morphology may vary from white to yellow or orange depending on the age of the culture, strain characteristics, and growth conditions. These phenotypic traits provide preliminary clues for identification, which are then confirmed through biochemical testing. Coagulase testing, either tube or slide-based, is widely used to distinguish *S. aureus* from coagulase-negative staphylococci, while commercial identification systems such as the API Staph system provide additional biochemical profiles for accurate species determination (Faires *et al.*, 2023; Paterson *et al.*, 2022).

Detection of methicillin resistance is primarily achieved by targeting the mecA and mecC genes, which encode penicillin-binding protein 2a (PBP2a), conferring resistance to β-lactam

antibiotics. Polymerase chain reaction (PCR) assays targeting these genes are considered the "gold standard" for confirming methicillin resistance due to their high specificity and sensitivity. Complementary phenotypic tests, including PBP2a latex agglutination and antibiotic susceptibility assays such as disk diffusion, agar screening, and minimum inhibitory concentration (MIC) determinations, are essential for assessing the functional expression of resistance and guiding therapeutic interventions. These phenotypic assessments also help detect heteroresistant populations that may not be apparent through molecular testing alone (Wendlandt *et al.*, 2022; Faires *et al.*, 2023).

Molecular typing methods have become indispensable for epidemiological investigations, enabling the differentiation of MRSA strains, tracing of outbreaks, and understanding of transmission dynamics between animals, humans, and the environment. Techniques such as multilocus sequence typing (MLST), staphylococcal cassette chromosome mec (SCCmec) typing, spa typing, and pulsed-field gel electrophoresis (PFGE) provide complementary information about strain relatedness, clonal complexes, and resistance gene dissemination. While some isolates may remain untypeable using a single approach, combining multiple typing methods enhances resolution and accuracy, particularly for lineages like CC398, which are commonly associated with livestock and may be poorly resolved by PFGE alone. These typing strategies are critical not only for outbreak investigations but also for designing targeted infection control measures and for monitoring the emergence of novel or hypervirulent MRSA clones in veterinary and household settings (Wendlandt *et al.*, 2022; Smith *et al.*, 2022; Paterson *et al.*, 2022).

The integration of culture-based, biochemical, molecular, and phenotypic methods ensures robust and accurate diagnosis of MRSA in animals. Early and precise identification allows veterinarians to implement appropriate antimicrobial therapy, reduce the risk of complications, and prevent transmission to other animals or humans. Moreover, accurate

diagnosis contributes to the broader understanding of MRSA epidemiology, informs risk assessment for zoonotic transmission, and supports public health interventions designed to mitigate the spread of this multidrug-resistant pathogen in both community and clinical environments (Abraham *et al.*, 2023; Faires *et al.*, 2023).

2.20 Treatment

The treatment of Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections in animals requires careful consideration of antimicrobial susceptibility patterns, as resistance profiles vary widely among strains. All MRSA isolates are intrinsically resistant to penicillins, cephalosporins, and other β-lactam antibiotics, including ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, and carbapenems, regardless of in vitro susceptibility results. This intrinsic resistance necessitates the use of alternative antimicrobial agents and underscores the importance of performing susceptibility testing prior to initiating therapy (Abraham *et al.*, 2023; Faires *et al.*, 2023). Resistance patterns are further complicated by host species and strain origin. For instance, livestock-associated MRSA CC398 isolates frequently exhibit resistance to tetracyclines, macrolides, and trimethoprim-sulfonamides, whereas human-associated strains show variable resistance profiles depending on geographic location, antimicrobial exposure history, and previous therapeutic interventions (Paterson *et al.*, 2022; Smith *et al.*, 2022). Understanding these differences is essential for the rational selection of effective therapies in both companion and farm animals.

Therapeutic strategies are tailored to the type and severity of the infection. Systemic infections, such as bacteremia, osteomyelitis, or septic arthritis, often necessitate prolonged administration of antimicrobials with proven activity against MRSA, guided by susceptibility results. In contrast, superficial or localized infections, such as skin abscesses, post-operative wound infections, or dermatitis, may respond adequately to topical antiseptic treatments including chlorhexidine, povidone-iodine, or antimicrobial ointments, particularly when used

in combination with systemic therapy for refractory cases (Abraham *et al.*, 2023). Surgical intervention may also be required, including drainage of abscesses, debridement of necrotic tissue, or removal of infected implants and catheters, as mechanical management complements antimicrobial therapy and reduces bacterial load (Jones *et al.*, 2022).

Combination therapies are often employed in cases where single-agent treatment proves insufficient. For example, oral doxycycline may be combined with rifampicin, or fusidic acid used alongside topical chlorhexidine, to achieve synergistic effects and reduce the likelihood of resistance development. The success of such combinations depends not only on host factors, such as immune competence and age, but also on environmental management, hygiene practices, and adherence to treatment protocols (Faires *et al.*, 2023; Wendlandt *et al.*, 2022). Critically important antimicrobials for human medicine, including vancomycin and linezolid, are generally reserved to prevent the emergence of strains capable of crossing species barriers. The use of these drugs in animals is controversial, as indiscriminate application could select for resistant strains with serious public health implications (Smith *et al.*, 2022).

Veterinary treatment protocols therefore emphasize antimicrobial stewardship, careful monitoring of clinical response, and adjunctive measures such as meticulous wound care, environmental disinfection, and isolation of infected or colonized animals. Despite appropriate therapy, some animals may remain asymptomatic carriers, serving as reservoirs for continued transmission within households, farms, or clinical settings. This highlights the need for ongoing surveillance, preventive measures, and education of animal owners and veterinary staff to limit the spread of MRSA. Case reports and controlled studies have demonstrated that multifaceted approaches, combining systemic and topical therapy with strict environmental control measures, are most effective in managing MRSA infections in

companion animals and livestock, reducing recurrence and minimizing zoonotic risk (Jones *et al.*, 2022; Abraham *et al.*, 2023; Paterson *et al.*, 2022).

2.21 Prevention of Methicillin-Resistant *Staphylococcus aureus*

Preventive strategies for Methicillin-Resistant *Staphylococcus aureus* (MRSA) in animals are aimed at interrupting the chain of transmission between humans and pets, as well as minimizing spread among animals in clinical, household, and farm environments. Fundamental to these strategies is maintaining rigorous hygiene practices, including thorough handwashing, the use of disinfectants on surfaces, and regular cleaning of animal living spaces. Environmental sanitation plays a critical role in reducing contamination of frequently touched surfaces, bedding, feeding areas, and grooming equipment, which can harbor MRSA for extended periods and act as reservoirs for transmission (Abraham *et al.*, 2023; Smith *et al.*, 2022). Veterinary clinics and hospitals represent high-risk settings, and strict infection control measures must be enforced. These include the use of disposable or easily laundered protective clothing, gloves, masks, and other barrier precautions during patient care, as well as the systematic disinfection of examination tables, instruments, and surgical suites (Paterson *et al.*, 2022).

Isolation of MRSA-infected or colonized animals is another essential component of prevention, reducing the risk of cross-contamination to other animals or humans. Wounds and surgical sites should be appropriately dressed, and contaminated materials, including dressings, gloves, and bedding, should be disposed of or disinfected safely. Screening high-risk animals, such as those admitted for surgery or animals from households with known MRSA-positive individuals, allows early identification of carriers and the implementation of targeted interventions. Routine surveillance programs in veterinary hospitals and boarding facilities further enhance the ability to detect outbreaks promptly, enabling timely containment measures and limiting the potential for wider dissemination (Faires *et al.*, 2023).

Decolonization of animals, although theoretically beneficial, is not routinely recommended due to the transient nature of MRSA colonization and the limited evidence supporting the consistent efficacy of antimicrobial regimens. When decolonization is considered necessary such as in cases of persistent carriage, animals in contact with high-risk humans, or recurrent clinical infections protocols may involve topical antiseptics, such as chlorhexidine washes, and in some instances, systemic antimicrobial therapy administered under strict veterinary supervision. These interventions should always be coupled with environmental cleaning, repeated screening, and behavioral adjustments to prevent re-exposure and reinfection (Wendlandt *et al.*, 2022; Abraham *et al.*, 2023).

Biosecurity measures play a pivotal role in the broader context of MRSA prevention. Limiting contact between MRSA-positive and susceptible animals, controlling visitor access to animal facilities, and ensuring staff and pet owners adhere to strict hygiene protocols are key strategies in reducing transmission risk. Enhanced environmental management, including frequent disinfection of kennels, cages, feeding areas, and bedding, reduces bacterial persistence in animal habitats. Equally important is education of pet owners, veterinary personnel, and farm workers about the zoonotic potential of MRSA, the importance of personal hygiene, early recognition of clinical signs, and the adoption of preventive measures in everyday animal care routines (Smith *et al.*, 2022; Paterson *et al.*, 2022; Faires *et al.*, 2023).

The combination of personal hygiene, environmental management, isolation protocols, risk-based screening, and targeted educational programs represents a multifaceted approach that is most effective in preventing MRSA colonization and infection. Implementing these measures consistently across household, veterinary, and farm settings not only protects animal health but also significantly reduces the risk of zoonotic transmission to humans, highlighting the interconnectedness of animal and public health in controlling MRSA (Pantosti & Giufrè, 2023).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The cross-sectional study was conducted in Ekosodin, a community situated within the University of Benin environs in Benin City, Edo State, Nigeria. Benin City, the capital of Edo State, is positioned at longitude 5.630 and latitude 6.340, with an estimated population of about 1,125,058 people. Ekosodin is a densely populated area within Benin City, predominantly inhabited by students and young individuals. The community's demographic and socio-economic characteristics facilitate high human-animal contact, particularly with owned and stray dogs which makes it a relevant location for the study.

3.2 Sample Size

The sample size was calculated according to the following formula:

$$n = \frac{Z^2 P (1 - P)}{d^2}$$

(Naing *et al.*, 2016).

Where:

n = required sample size

Z = confidence level at 95% (standard value of 1.98).

P = estimated prevalence rate (as obtained from literature review).

d = margin of error at 5% (standard value = 0.05).

The prevalence rate from previous literature review was 13% prevalence rate (Loeffler *et al.*, 2011)

Substituting values for *Staphylococcus aureus*;

$$n = \frac{1.96^2 \times 0.13 (1-0.2)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.13 \times (0.8)}{0.0025}$$

n = 160 sample sizes

Therefore 160 nasal samples from dogs were used for the study.

3.3 Inclusion Criteria

Apparently healthy dogs that have not been on antibiotics for the past two weeks were included in this research

3.4 Exclusion Criteria

Dogs that are sick or have been on antibiotics medication were excluded in this research

3.5 Ethical Approval

Ethical approval for the study was obtained from the Edo State Ministry of Health prior to commencement of any research activities with a reference number HA/737/25/D/06180726. The study protocol was reviewed to ensure compliance with ethical standards that protect the rights, safety, and well-being of both the animals involved and the community at large. Consent was sought from dog owners within Ekosodin Community before sample collection, ensuring that they are fully informed about the purpose, procedures, risks, and benefits of the study. Confidentiality and privacy of the participants and their pets were maintained throughout the research process.

3.6 Collection and Processing of Samples

From a total of 160 apparently healthy dogs who had not taken antibiotics in the last two weeks, swab samples were collected from their nostrils. The swabs were cultured onto Mannitol Salt Agar (MSA), which is selective for *Staphylococcus* species due to its high salt concentration. The plates was incubated at 37°C overnight. Yellow coloration of colonies on MSA indicated mannitol fermentation, which is characteristic of *Staphylococcus aureus*. Isolates was then further identified using standard microbiological techniques.

3.7 Identification of Isolates

All isolates were identified using conventional methods such as Colonial Morphology, Gram Stain and appropriate biochemical tests namely catalase, coagulase (slide and/or tube).

3.8 Antimicrobial Susceptibility Testing

The antibiotic susceptibility testing was determined using Kirby – Bauer disc diffusion method (Bauer and Kirby; 1996) on Mueller-Hinton. Test organism (*Staphylococcus aureus*) was emulsified in sterile water and the turbidity matched with 0.5 McFarlard standards. Once matched, a sterile cotton wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on the side of the test tube. The entire surface of the Mueller-Hinton agar plate was seeded by swabbing in three directions with the swab. The antibiotics were placed on the plate with the use of a sterile forceps. The antibiotics to be used included the following: Cefoxitin (30µg), Cefuroxime (30µg), Ofloxacin (5µg), Erythromycin (15µg), Gentamycin (10µg), Azithromycin (15µg), Amoxicillin clavulanate (30µg), Cefotaxime (25µg), Ceftriaxome sulbactam (45µg), Cefixime (5µg), Levofloxacin (5µg), Ciprofloxacin (5µg).

3.9 Detection of Methicillin Resistant *Staphylococcus aureus*

Staphylococcus species isolated were screened for methicillin-resistance by following CLSI guidelines using 30µg of cefoxitin discs (Abtek U.K) (CLSI, 2013). The plates were read after incubation at 37^{o}C overnight. Zone diameter ≤ 21mm was considered cefoxitin resistant and a zone diameter greater than 21mm was considered sensitive.

3.10 Molecular (Plyrothypine) Identification of MRSA

Molecular identification of MRSA isolates was performed to confirm the presence of the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a) responsible for methicillin resistance. DNA was extracted from confirmed *S. aureus* isolates using a boiling method. Briefly, a loopful of bacterial colony was suspended in 200 µL of sterile distilled water,

boiled at 100°C for 10 minutes, and centrifuged at 10,000 rpm for 5 minutes. The supernatant containing the DNA was transferred into a sterile microtube and stored at −20°C until use.

Polymerase Chain Reaction (PCR) was carried out using specific primers for the *mecA* gene. The reaction mixture (25 µL) contained 12.5 µL of PCR master mix, 1 µL each of forward and reverse primers, 2 µL of DNA template, and 8.5 µL of nuclease-free water. Amplification was carried out under the following cycling conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes (Zhang et al., 2022).

The amplified products were visualised by electrophoresis on 1.5% agarose gel stained with ethidium bromide, and the bands were viewed under UV transillumination. A 310 bp band indicated the presence of the *mecA* gene, confirming MRSA (Anand et al., 2019; Goudarzi et al., 2020).

3.11 Statistical Analysis

Data was entered into Microsoft Excel and analysed using IBM SPSS Statistics (version 28 or latest). Descriptive statistics such as frequencies and percentages were used to summarise the prevalence of Staphylococcus aureus and MRSA, as well as their antibiotic susceptibility patterns. The Chi-square (χ^2) test was used to assess the associations between MRSA prevalence and variables like age and gender. A p-value <0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

This chapter presents the findings from the study on the prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from the nostrils of dogs in Ekosodin, Benin City, Edo State, Nigeria. The results were obtained from 160 dogs sampled, and the proportions have been maintained to reflect the practical outcomes.

4.1 Presentation of Result

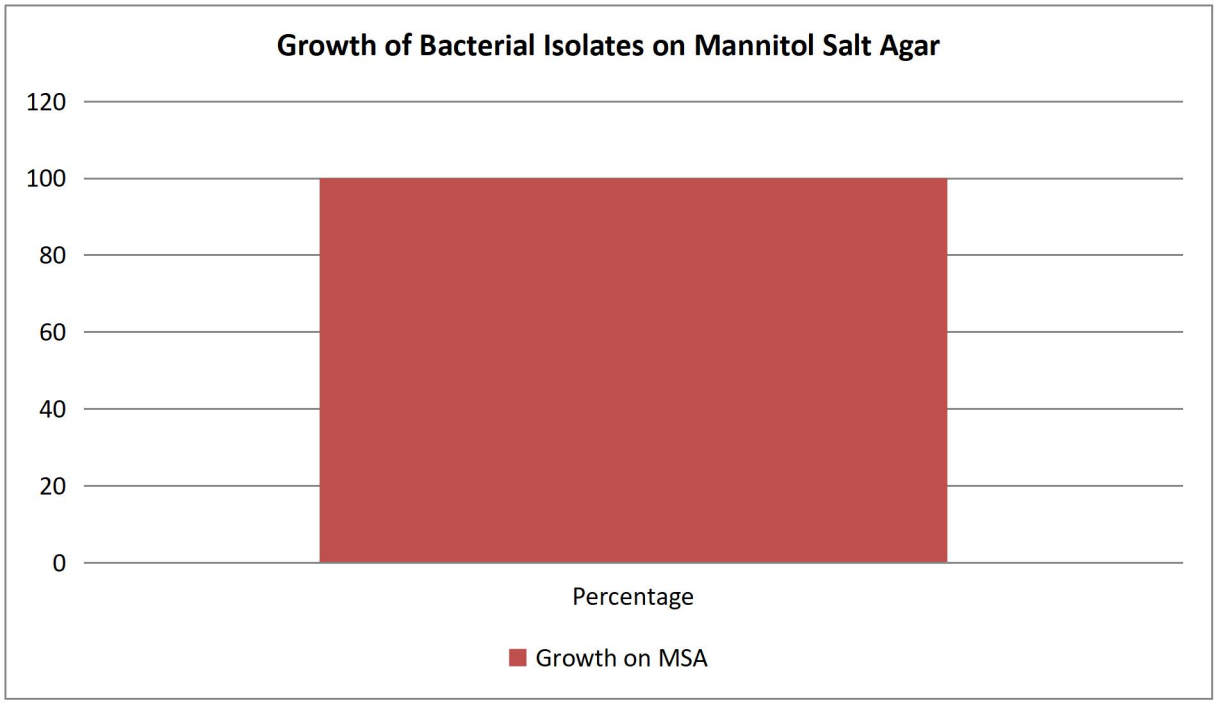

Figure 4.1: Growth of bacterial isolates on Mannitol Salt Agar (MSA)

Figure 4.1 shows that all 160 nasal swab samples (100%) showed growth on Mannitol Salt Agar, indicating the presence of salt-tolerant *Staphylococcus* species.

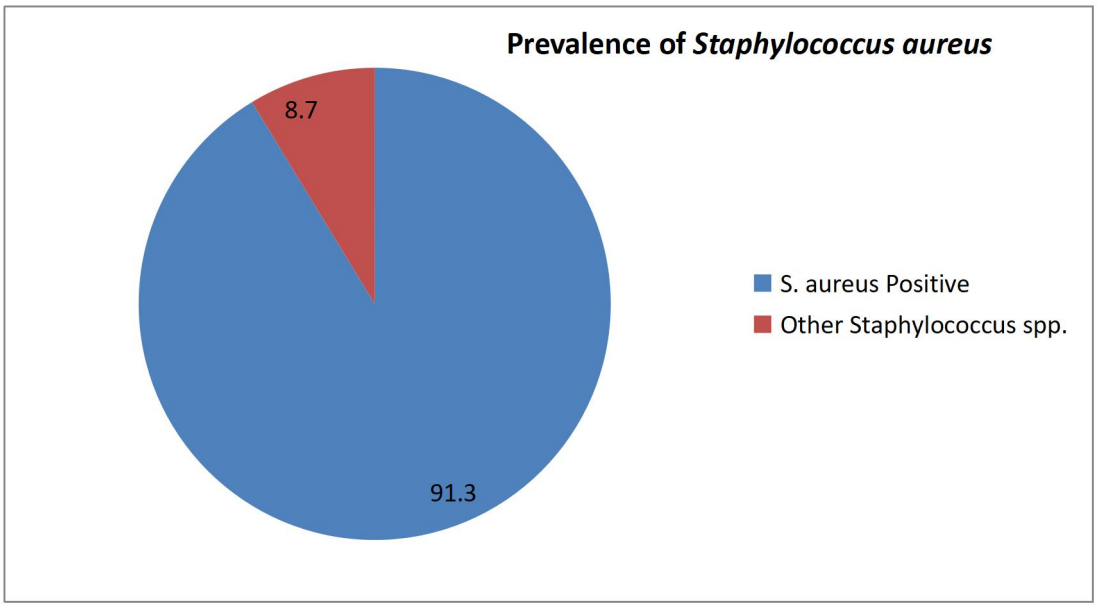

Figure 4.2: Prevalence of *Staphylococcus aureus* isolates

Figure 4.2 shows that out of the 160 dogs sampled, 146 (91.3%) were confirmed to carry *Staphylococcus aureus*. The remaining 14 (8.7%) carried other *Staphylococcus* species.

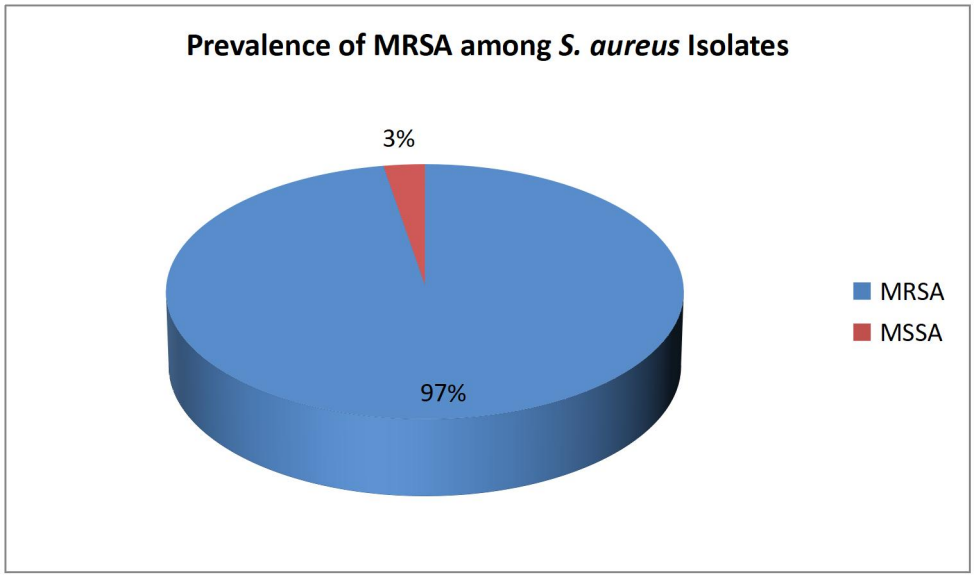

Figure 4.3: Prevalence of MRSA among *S. aureus* isolates

Figure 4.3 shows that of the 146 *S. aureus* isolates, 142 (97.3%) were resistant to cefoxitin, indicating MRSA. This corresponds to an overall MRSA prevalence of 88.8% (142/160) in the total dog population sampled.

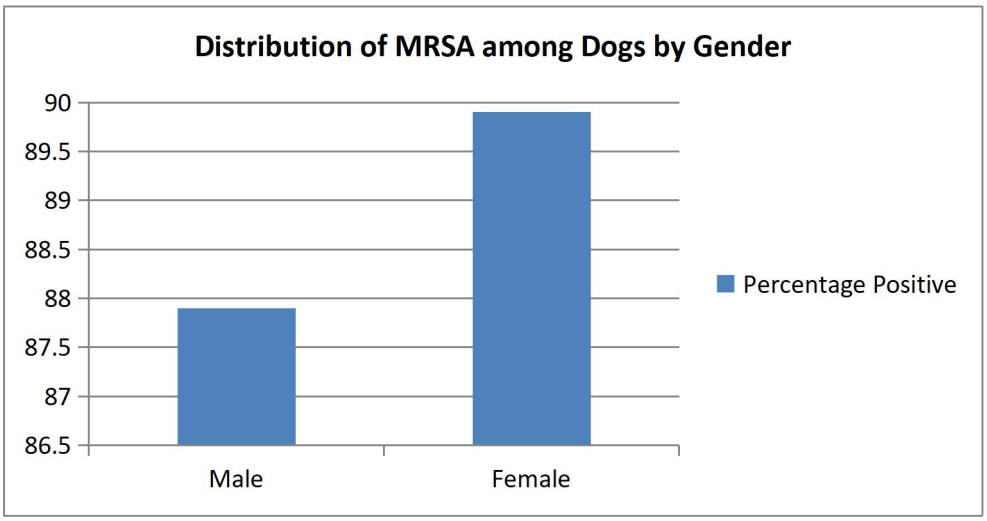

Figure 4.4: Distribution of MRSA among Dogs by gender

Figure 4.4 shows that MRSA prevalence was high in both male (87.9%) and female (89.9%) dogs, with no statistically significant difference ($p > 0.05$).

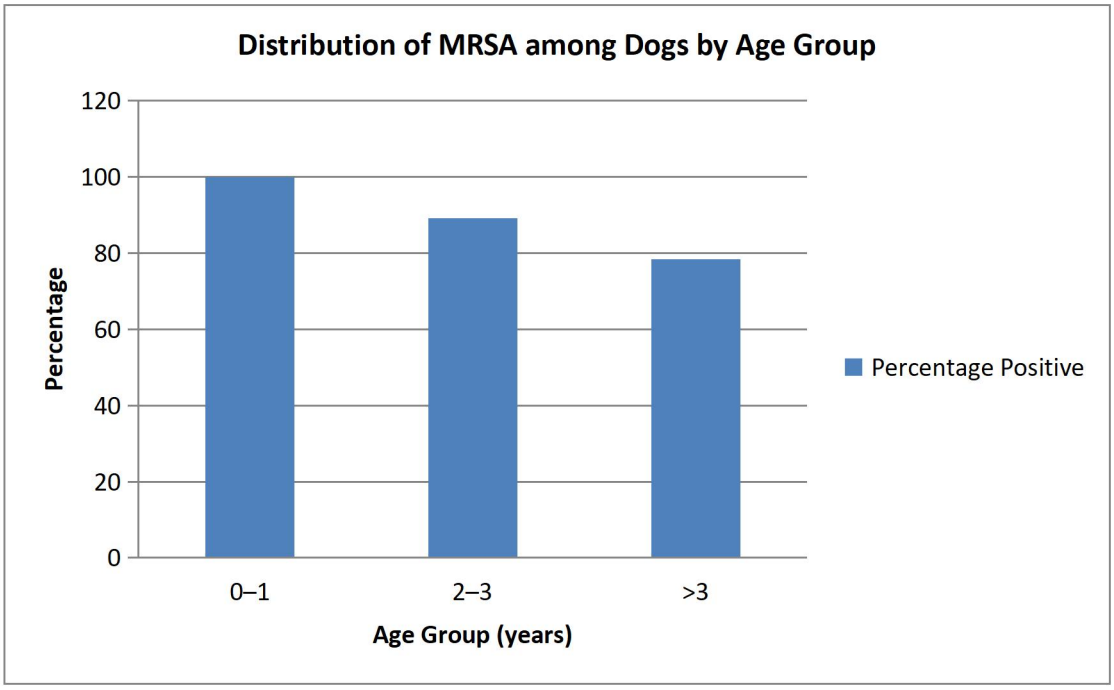

Figure 4.5: Distribution of MRSA among Dogs by age group

Table 4.5 shows that MRSA prevalence was highest among dogs aged 0–1 years (100.0%), followed by those aged 2–3 years (89.2%), and the lowest in dogs over 3 years (78.4%). The difference between age groups was not statistically significant ($p > 0.05$).

Table 4.1: Antibiotic susceptibility of MRSA isolates

Antibacterial drugs	MRSA (%) n=43	MSSA (%) n=11	P value
Ciprofloxacin (CPX)	20 (46.5)	9 (81.8)	0.047
Erythromycin (E)	10 (23.3)	8 (72.7)	0.004
Levofloxacin (LEV)	22 (51.2)	10 (90.9)	0.019
Gentamicin (GN)	18 (41.9)	7 (63.6)	0.310
Cefuroxime (CEF)	5 (11.6)	6 (54.5)	0.005
Rifampicin (RD)	25 (58.1)	9 (81.8)	0.181
Ceftazidime (CTZ)	7 (16.3)	5 (45.5)	0.053
Streptomycin (S)	15 (34.9)	6 (54.5)	0.305
Azithromycin (AZM)	8 (18.6)	4 (36.4)	0.237
Amoxicillin (AMX)	0 (0.0)	0 (0.0)	1.000

All the nasal swab samples collected from the dogs showed bacterial growth on Mannitol Salt Agar, confirming that the isolates were salt-tolerant *Staphylococcus* species. A very high proportion of the samples were confirmed to be *Staphylococcus aureus*, indicating that this organism is commonly present in the nasal cavity of dogs in the study area (Figure 4.1 and Figure 4.2).

Among the *S. aureus* isolates, the majority were methicillin-resistant, representing almost all of the confirmed *S. aureus* samples. This shows that MRSA is highly prevalent among the dog population in Ekosodin, with nearly nine out of every ten dogs harbouring MRSA (Figure 4.3).

When analysed by gender, both male and female dogs showed similarly high levels of MRSA carriage. The slight differences observed were not statistically significant, meaning that sex did not play a major role in MRSA prevalence (Figure 4.4).

In terms of age distribution, younger dogs, particularly those less than one year old, had the highest prevalence, followed by dogs aged two to three years, while those above three years had a comparatively lower prevalence. Although these variations suggest that age may influence carriage rates, the differences were not statistically significant (Figure 4.5).

The results from the antibiotic susceptibility profile of the isolates showed that methicillin-sensitive *S. aureus* (MSSA) were more responsive to most of the antibiotics tested compared to MRSA isolates. Statistically significant differences were observed for ciprofloxacin, erythromycin, levofloxacin, and cefuroxime, where MSSA demonstrated greater susceptibility (Table 4.1).

MRSA isolates, however, retained partial sensitivity to rifampicin and some fluoroquinolones, though resistance levels remained high overall. Complete resistance was observed against amoxicillin in both MRSA and MSSA isolates. These findings highlight the challenges in antibiotic therapy for canine *S. aureus* infections in the study area, especially when dealing with MRSA.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Staphylococcus aureus is a versatile opportunistic pathogen capable of causing a wide range of infections in both humans and animals. The emergence of methicillin-resistant *S. aureus* (MRSA) has remained a global concern because of limited treatment options and its potential for zoonotic transmission. Companion animals, especially dogs, have been increasingly recognised as important reservoirs of MRSA, making their role in public health highly significant.

In this study, all nasal swab samples collected from dogs in Ekosodin showed growth on Mannitol Salt Agar, confirming the presence of salt-tolerant staphylococci. A high proportion (91.3%) were confirmed as *S. aureus*, and 97.3% of these were methicillin-resistant, giving an overall MRSA prevalence of 88.8%. This prevalence is considerably higher than what has been reported in other regions. For instance, Sharma *et al.* (2020) reported a lower MRSA prevalence of 28% among companion dogs in India, while Rodríguez-Lázaro *et al.* (2021) found 36% in Spain. The differences could be explained by variations in antibiotic use policies, levels of veterinary care, and environmental exposure between regions. The high prevalence in Ekosodin may also reflect frequent dog-to-dog interactions, limited veterinary diagnostic services, and possible human-to-animal transmission within households, which aligns with findings by Gómez-Sanz *et al.* (2020), who highlighted the role of close human-animal contact in MRSA carriage.

The study also revealed that gender did not significantly influence MRSA prevalence, with almost equal rates in male and female dogs. This is consistent with the findings of Weese *et al.* (2021), who reported no sex-related difference in MRSA carriage in Canadian dogs. The

independence of MRSA prevalence from sex suggests that other factors, such as environment, antibiotic exposure, and host immunity, may play more significant roles.

When age distribution was considered, younger dogs aged 0–1 year showed the highest prevalence (100%), followed by those aged 2–3 years (89.2%), while dogs older than three years had lower prevalence (78.4%). This agrees with the observations of Alzahrani *et al.* (2022), who noted higher MRSA colonisation in younger animals, attributing it to immature immune responses and frequent exposure in communal or outdoor settings. However, in contrast, Hassan *et al.* (2023) reported no significant age-related differences in MRSA prevalence among dogs in Egypt. This discrepancy may be due to differences in study populations, sample sizes, or animal management practices.

The antimicrobial susceptibility testing revealed that MRSA isolates exhibited multidrug resistance, with complete resistance to amoxicillin and reduced susceptibility to macrolides and aminoglycosides. MSSA isolates, on the other hand, showed higher susceptibility to fluoroquinolones and cefuroxime. These results are consistent with the findings of El-Deeb *et al.* (2020) in Saudi Arabia, who reported high levels of resistance to beta-lactams and macrolides among MRSA isolates from dogs. Similarly, Odetokun *et al.* (2020) observed widespread resistance among MRSA isolates in Nigeria, linking it to misuse and over-the-counter access to antibiotics. Differences in susceptibility patterns between MSSA and MRSA reflect the genetic acquisition of resistance determinants such as the mecA gene, which confers beta-lactam resistance (Larsen *et al.*, 2022).

The high level of resistance observed in this study suggests that indiscriminate and unregulated antibiotic use is a major driver of resistance in the study area. In many low- and middle-income countries, limited access to veterinary diagnostics, economic constraints, and improper prescription practices create conditions for selective pressure that favour resistant strains (Moges *et al.*, 2021). The close interaction between dogs and humans in Ekosodin

further increases the risk of zoonotic transmission, which echoes the conclusions of Abd El-Ghany *et al.* (2021) that MRSA in pets can serve as a bridge for resistant bacteria between animals and humans.

The findings of this study confirm that MRSA is highly prevalent among dogs in Ekosodin, with resistance patterns that mirror global trends, but with prevalence rates higher than those reported in many other parts of the world. This suggests the urgent need for improved antibiotic stewardship, enhanced diagnostic capacity, and community education on the risks of zoonotic transmission.

5.2 Conclusion

This study revealed an alarmingly high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among dogs in Ekosodin, Benin City, with an overall carriage rate of 88.8%. Younger dogs were more frequently colonised, while gender did not significantly influence prevalence, indicating that age-related immune maturity rather than sex is a more important factor in susceptibility.

The antimicrobial susceptibility profile showed extensive multidrug resistance among MRSA isolates, with only partial sensitivity retained to rifampicin and fluoroquinolones such as levofloxacin and ciprofloxacin. Complete resistance to amoxicillin and high resistance to macrolides and aminoglycosides emphasise the limited treatment options available.

These findings underline a serious public health concern, as dogs may serve as reservoirs of resistant strains that could spread to humans through close contact. The results highlight the urgent need for prudent antibiotic use, improved veterinary diagnostic capacity, and strict infection control measures to curb the emergence and transmission of MRSA in both animals and humans.

5.3 Recommendations

1. Government and veterinary health authorities should prioritise the monitoring of MRSA prevalence in companion animals, particularly in high-risk communities.
2. Dog owners should be educated on proper hygiene practices, limiting unnecessary contact, and ensuring regular veterinary check-ups.
3. Indiscriminate use of antibiotics in veterinary practice should be discouraged, and susceptibility testing should be conducted before initiating treatment.
4. Public awareness campaigns should highlight the potential risk of MRSA transmission from animals to humans.
5. Comprehensive surveillance and control measures should be implemented to curb the spread of MRSA in both animal and human populations.

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APPENDIX I

REQUEST ETHICAL APPROVAL

Department of Medical Laboratory Science,
School of Basic Medical Sciences,
University of Benin,
Benin City.
12th May, 2025.

The Honourable Commissioner,
Edo State Ministry of Health,
Edo State.

Dear Sir,

APPLICATION FOR ETHICAL APPROVAL

I am a 500 Level student of the above named department and I humbly apply for ethical approval to carry out a research entitle "Prevalence of methicillin resistant *Staphylococcus Aureus* (MRSA) isolated from dogs nostrils in Ekosodin Benin City, Edo State, Nigeria". The purpose of this research is to determine the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from dogs nostrils in Ekosodin, Benin City, Edo State, Nigeria.

Investigators: Dr (Mrs) Zainab Omoruyi

Uba Success Nneoma

Attached with this letter is a copy of the research proposal for your perusal.

I hope I am granted approval because I strongly believe the results from this research will greatly contribute to the progress of this great state. Thank you for your time and consideration.

Yours faithfully,

_____

Uba Success Nneoma
Principal Investigator
Tel: +234-916-281-4296

APPENDIX III

ETHICAL APPROVAL

EDO STATE MINISTRY OF HEALTH
HEALTH RESEARCH ETHICS COMMITTEE

PROTOCOL NUMBER	**HA/737/25/D/05230726 (PLEASE QUOTE IN ALL ENQUIRIES)**
APPROVAL NUMBER	**HA/737/25/D/06180726**
TITLE OF RESEARCH PROPOSAL	**PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ISOLATED FROM DOGS' NOSTRILS IN EKOSODIN BENIN CITY, EDO STATE, NIGERIA**
PRINCIPAL INVESTIGATOR (S)	**UBA SUCCESS NNEOMA**
DATE CONSIDERED	**18TH JUNE, 2025.**
DECISION OF THE COMMITTEE	**APPROVED**

THIS APPROVAL DATES 18/06/2025 TO 18/06/2026. IF THERE IS A DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC EDO SMoH SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK: Please kindly note that the HREC Edo SMoH seal authenticates this approval

DR (MRS.) OMONYEMEN B. BELLO (MBBS, MPH, FPHCM) *(CHAIRMAN)* **SIGNATURE & DATE**.....23/6/25.....

SUPERVISOR(S) .....

.....

ATTESTATION BY INVESTIGATOR(S)

No participant accrual or activity related to this research may be conducted outside of the approval dates. All informed consent forms used in this study must carry the Edo SMoH HREC-assigned number and duration of your research. No changes are permitted in the research without prior approval of the Edo SMoH HREC except in circumstances outlined in the Code. The Edo SMoH HREC reserves the right to conduct compliance visits to your research site without previous notification.

Signature & Date.....

Original copy collected by me.

edohrec@edostate.gov.ng

Room 16, Block D, 2nd floor, State secetariat building.

APPENDIX III

INFORMED CONSENT

Department of Medical Laboratory Science,
School of Basic Medical Sciences,
University of Benin,
Benin City.

Dear Dog Owner,

LETTER OF INFORMED CONSENT

I am a final year student of Medical Laboratory Science at the University of Benin, conducting a research study as part of the requirements for the completion of my Bachelor's degree. The study aims to determine the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in dogs' nostrils in Ekosodin.

You are being invited to participate by allowing a non-invasive nasal swab sample to be collected from your dog. This sample will be analyzed in the laboratory to detect the presence of MRSA bacteria.

Your Rights and Assurances:

- Participation is entirely voluntary.
- You may choose to withdraw at any time without any consequences.
- All information and results obtained will be treated with the strictest confidentiality.
- No harm will come to your dog during the sample collection process.
- There are no costs to you, and no financial benefit from participating.

Your consent confirms that you understand the purpose of this study and agree to allow your dog to participate in the sampling.

CONSENT STATEMENT

I, _____, am the legal owner or caretaker of the dog named _____. I have read and understood the nature of the research and give my **informed consent** for my dog to be involved in this study.
Signature: _____
Date: _____
Phone Number: _____

Thank you for your participation.

Sincerely,

Uba Success Nneoma
+234 916 281 4296
Researcher
University of Benin

APPENDIX IV

QUESTIONNAIRE

DEPARTMENT OF MEDICAL LABORATORY SCIENCE
SCHOOL OF BASIC MEDICAL SCIENCES
UNIVERSITY OF BENIN
BENIN CITY, EDO STATE, NIGERIA

Dear Respondent

I am an undergraduate student of the above-named institution. I am conducting research on "Prevalence of methicillin resistant *Staphylococcus Aureus* (MRSA) isolated from dogs nostrils in Ekosodin Benin City, Edo State, Nigeria". This questionnaire is designed to collect information for the study. Kindly respond to the following questions. Please tick (√) as required in one or more places or write in the space provided. All questions and responses are very important to this study, please do well to answer them appropriately.

Thanks.

Uba Success Nneoma
Researcher

SECTION A: What is the prevalence rate of MRSA among dogs in Ekosodin?

S/N	ITEMS	YES	NO
1.	Has your dog ever been tested for bacterial infections?		
2.	Has your dog ever been diagnosed with *Staphylococcus aureus* or MRSA?		
3.	Has your dog experienced repeated skin infections or abscesses?		
4.	Does your dog regularly visit a veterinary clinic?		
5.	Has a veterinarian ever suggested testing your dog for MRSA?		

SECTION B: Is there a difference in MRSA prevalence between domestic and stray dogs?

S/N	ITEMS	YES	NO
6.	Is the dog a domestic (household) pet?		
7.	Does the dog have regular interaction with other animals?		
8.	Does the dog roam freely without restriction?		
9.	Does the dog frequently interact with humans?		

10.	Is the dog regularly fed and groomed?		

SECTION C: Are there risk factors associated with MRSA colonization in dogs?

S/N	ITEMS	YES	NO
11.	Is the dog older than 3 years?		
12.	Does the dog reside in a crowded or urban environment?		
13.	Has the dog undergone surgery or had open wounds recently?		
14.	Is the dog frequently exposed to refuse or waste dumps?		
15.	Are there multiple pets or animals in the same household?		

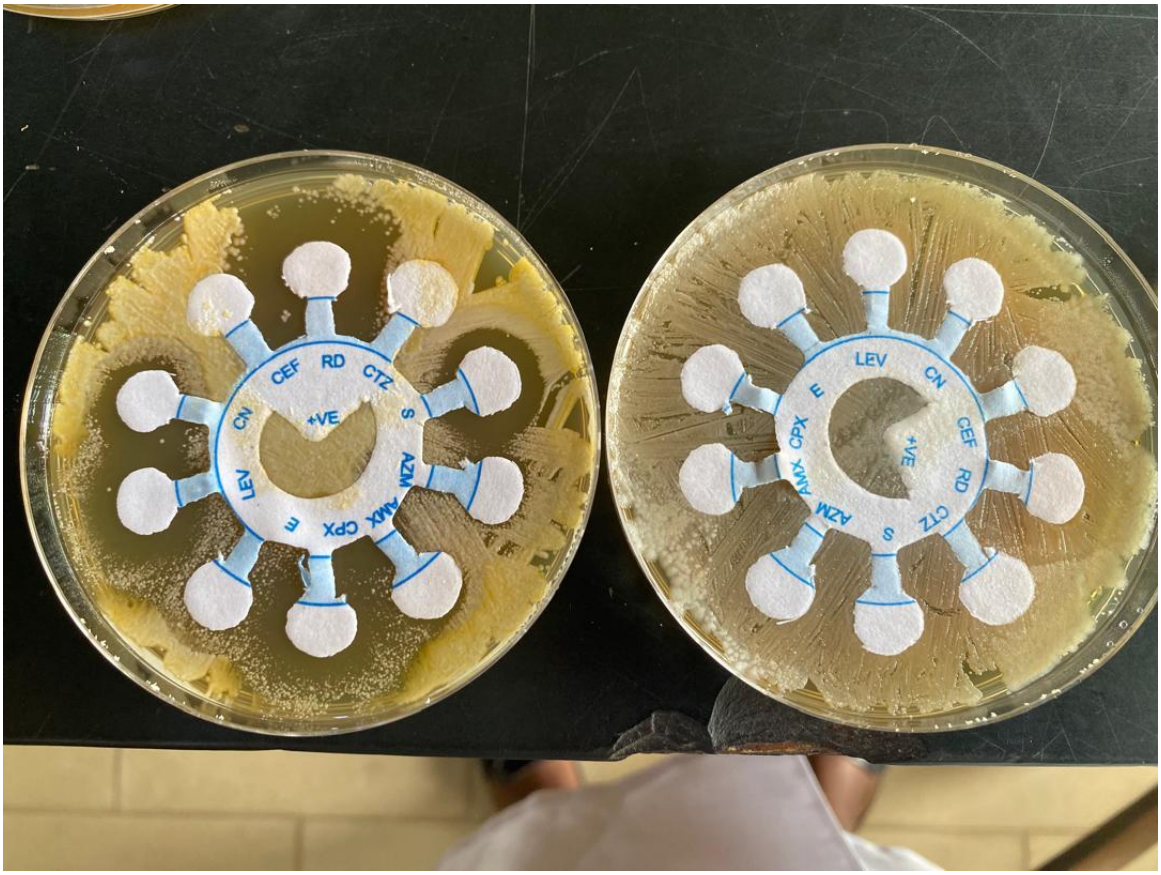
APPENDIX V

ANTIBACTERIAL AGENT

The antibacterial susceptibility discs to be used for this study was purchased from reputable Pharmacy stores in Lagos, these include:

i. Gram Positive Multidisc
ii. Imipenem(10µg)
iii. Cefuroxime(30µg)
iv. Ofloxacin (5µg)
v. Erythromycin (15µg)
vi. Gentamycin(10µg)
vii. Azithromycin(15µg)
viii. Amoxicillin clavulanate(30µg)
ix. Cefotaxime(25µg)
x. Ceftriaxome sulbactam (45µg)
xi. Cefixime (5µg)
xii. Levofloxacin(5µg)
xiii. Ciprofloxacin(5µg)
xiv. Cefoxitin(30µg) disc.

APPENDIX VI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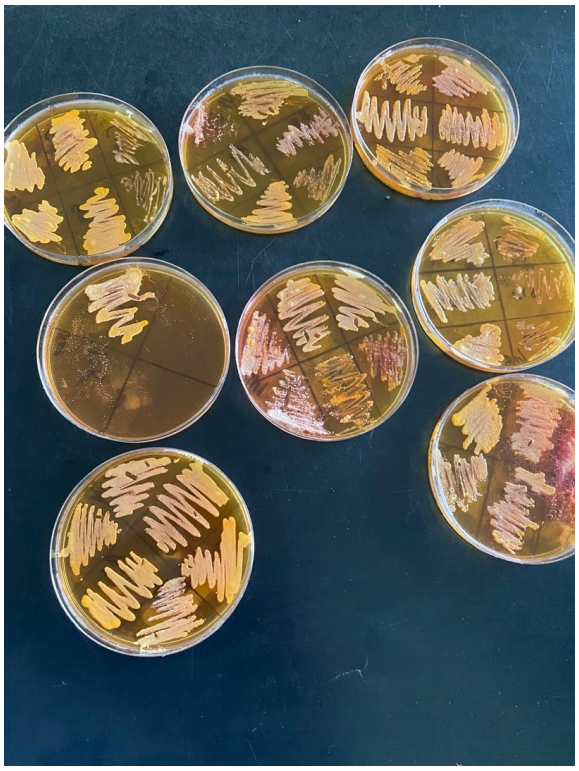


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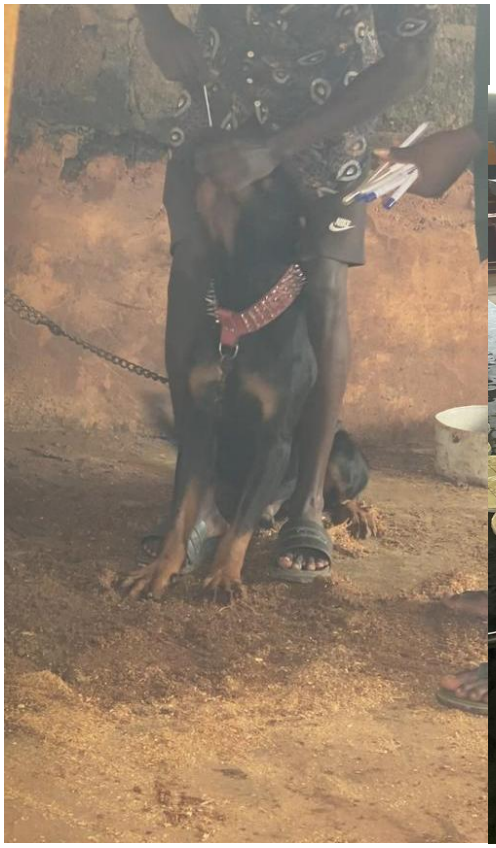


PMB