Anti-Microbial Susceptibility of *Staphylococcus aureus* in Paediatric Patients in a Tertiary Hospital in Benin City, Nigeria.

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (B.Sc) IN MICROBIOLOGY.

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CERTIFICATION

This is to certify that this work was carried out by **Sarah Esosa OSAGIEDE (MISS)** with matriculation number **LSC2104007** of the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin city. Nigeria.

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Mr E. C. Wemambu **DATE**

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HEAD OF DEPARTMENT

DEDICATION

The content of this research is dedicated to God Almighty for sustaining me till now.

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I can't cease to be grateful to GOD ALMIGHTY for all He has done in the course of this program.

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ABSTRACTS

Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi, and parasites no longer respond to antimicrobial agents. As a result of drug resistance, antibiotics (usually used for bacteria) and other antimicrobial agents become ineffective, and infections become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability, and death. This study comprehensively investigated the antimicrobial susceptibility patterns of *Staphylococcus aureus* isolated from paediatric patients. The study adopted a cross-sectional epidemiological design. The participants of the study include paediatric patients aged 2–17 years within UBTH with clinically diagnosed bacterial infections or paediatric patients who were suspected to eventually receive antibiotic treatments. A total of 53 samples were collected from this population, using rectal and nasal swabs, these samples were cultured on MacConkey agar and mannitol salt agar respectively. Isolates were characterized using conventional cultural techniques. The findings of the study showed that the mean age of the population is between 30.5-117.5 months, With male gender being predominant in the study. A total of 53 *Staphylococcus* isolates were identified, comprising 36(67.9%) *S. aureus* and 17(32.1%) Coagulase negative *Staphylococcus aureus* (CoNS). The antimicrobial susceptibility profile of *Staphylococcus aureus* isolates, indicated a high level of susceptibility to majority of the antibiotics; including amikacin, amoxicillin-clavulanic acid, tigecycline, meropenem, levofloxacin, erythromycin, cefuroxime, and tetracycline. Resistance was most pronounced against cefoxitin, cefazidime, ciprofloxacin, erythromycin, and sulfamethoxazole-trimethoprim, where significant proportions of isolates were resistant (ranging from approximately 45% to 80%). Based on the findings of this study, it can be infer that there is mild-high range of AMR among paediatric patient in Benin City. The study thus, supports the need for necessary action, including rational drug use, continuous surveillance, and deployment of adequate preventive and curative policies and actions.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The human environment harbours trillions of microbes, including protists, bacteria, viruses, and fungi. These organisms constitute the human microbiome and are recognised for both their beneficial and adverse effects on human health (Ahn and Hayes, 2021). Microbes have evolved diverse strategies to colonise and invade human tissues, leading to severe diseases despite multiple host defence mechanisms (Ribert and Cossart, 2015). In such instances of disease onset in humans caused by microbes, clinical diagnostic procedures are carried out to identify the aetiology of the disease. Antimicrobials, including antibiotics, antivirals, antifungals, and anti-parasitic, are drugs generally used to prevent and treat infectious diseases caused by microbes in humans, animals, and plants (Yu *et al*., 2023). Antimicrobial medicines are the cornerstone of modern medicine, enhancing the health of humans and animals and extending their lifespan (WHO, 2019).

Overuse of antimicrobial drugs in clinical, veterinary, and agricultural contexts causes microbes to develop resistance and adaptive mutations (Qurbani *et al*., 2024). This allows them to survive in situations with high levels of antibiotics and antiseptics, which would otherwise kill them. Bacteria and other microorganisms may rapidly adapt, modify, and spread adaptive genetic components through horizontal gene transfer, resulting in different resistance mechanisms (Ahmed *et al*., 2024).

Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi, and parasites no longer respond to antimicrobial agents. As a result of drug resistance, antibiotics (usually used for bacteria) and other antimicrobial agents become ineffective, and infections become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability, and death (Yu

et al., 2023). Resistance may arise and spread among animals, people, plants, and the environment. While AMR is a natural process that occurs due to genetic changes in pathogens, it is hastened by avoidable human behaviour, including the misuse and abuse of antimicrobials, poor infection prevention and control, pollution, and poor sanitation, including inadequate solid waste, water, and wastewater management (Mitchell *et al*., 2023).

AMR is fundamentally a One Health issue: antibiotic usage and resistant organisms spread across humans, animals (livestock, aquaculture, companion animals), and the environment. Overuse in agriculture selects for resistant strains that can spread to humans through food chains, direct contact, or environmental contamination; conversely, human waste contaminating the environment can increase resistance among environmental bacteria, which can then transfer genes back to pathogens. Modern national AMR strategies therefore emphasise coordinated action across ministries of health, agriculture, and the environment, surveillance linking human and animal isolates, restrictions on non-therapeutic antibiotic use in animals, and investment in wastewater treatment and infection prevention (WHO, 2022).

AMR poses a threat to global health, food security, and achieving the 2030 Sustainable Development Goals (SDGs). It is estimated that bacterial AMR was directly responsible for 1.27 million deaths worldwide in 2019 and contributed to 4.95 million deaths (Antimicrobial Resistance Collaborators, 2019). Tackling AMR is critical to preserving the world's ability to treat diseases in humans, animals, and plants, reducing risks to food safety and security, and protecting the environment. However, children and neonates are highly vulnerable to the impact of antimicrobial resistance. Substantial neonates, children, and adolescents aged younger than 19 years (Bamford *et al*., 2024).

Nigeria has recognized AMR as a national priority and has taken several steps to mitigate its impact. The Second One Health National Action Plan (NAP 2.0, 2024–2028) was launched to build on the achievements and address the gaps identified in NAP 1.0 (2017–2022). The plan involves multiple ministries: Health, Agriculture, Environment, and Food Security and seeks to strengthen regulation, diagnostics, and stakeholder engagement (Federal Ministries of Agriculture, Environment, Health and Social Welfare, Federal Republic of Nigeria, 2024; WHO Regional Office for Africa, 2024).

The history of AMR traces back to the discovery of penicillin in 1928 by Alexander Fleming, Fleming (2001) and the subsequent mass production and utilisation of antibiotics in the 1940s. However, resistant organisms emerged almost immediately thereafter. The first cases of penicillin-resistant Staphylococcus aureus were reported in 1942, Rammelkamp and Maxon (1942), along with tetracycline resistance by 1953 (Knight and Holzer, 1954). The widespread agricultural use of antibiotics in the 1950s–1960s also accelerated resistance. The MRSA was reported in 1961, followed by resistance to multiple antibiotic classes (Jevons, 1961; Parker and Jevons, 1964). The 1980s saw a global epidemic of MDR tuberculosis (Steiner *et al*., 1964). In the 1990s, gram-negative pathogens such as *Escherichia coli* and *Klebsiella pneumonia* developed ESBL resistance (Jacoby and Han, 1996). The rise of MDR diminished the number of available effective antibiotics and resulted in the withdrawal of pharmaceutical companies from antibiotic research. This perfect storm of increasing resistance and lack of new drug development continues to strain healthcare systems today. We have now entered a dangerous post-antibiotic era where common infections and minor injuries can once again become lethal. If solutions are not urgently implemented, it is expected that millions of people may die annually from AMR

infections. Thus, this research evaluates the antimicrobial resistance in a Nigerian population, specifically Children.

Table 1.1: Key Resistant Pathogens in Children and Their Clinical Implications

Pathogen	Common Infections in Children	Major Resistance Mechanisms	Key Resistant Strains/Phenotypes	Clinical Implications	References
Staphylococcus aureus	Skin and soft tissue infections, pneumonia, sepsis, osteomyelitis	mecA gene encoding altered penicillin-binding protein (PBP2a)	Methicillin-resistant *S. aureus* (MRSA)	Limited treatment options; need for vancomycin or linezolid; prolonged hospital stays	WHO, 2024; CDC, 2023
Streptococcus pneumoniae	Otitis media, meningitis, bacteremia, pneumonia	Altered penicillin-binding proteins; efflux pumps	Penicillin- and macrolide-resistant *S. pneumoniae*	Reduced efficacy of β-lactams and macrolides; vaccine escape strains emerging	WHO, 2024
Escherichia coli	Urinary tract infections, neonatal sepsis, diarrhea	Extended-spectrum β-lactamase (ESBL) production; plasmid-mediated AmpC and carbapenemases	ESBL-producing *E. coli*; carbapenem-resistant *E. coli* (CRE)	Failure of empirical therapy; need for carbapenems or colistin	WHO, 2024; Federal Ministries of Health, 2024
Klebsiella pneumoniae	Neonatal sepsis, pneumonia, wound infections	ESBL and carbapenemase (NDM, KPC, OXA-48) production	Carbapenem-resistant *K. pneumoniae* (CRKP)	High morbidity and mortality in neonatal intensive care units; limited antibiotic options	WHO Regional Office for Africa, 2024
Pseudomonas aeruginosa	Hospital-acquired pneumonia, burn, and wound	Efflux pumps; porin loss; β-lactamase production	Multidrug-resistant *P. aeruginosa* (MDRPA)	Persistent infections in immunocompromised children: high treatment	WHO, 2024; CDC, 2023

	infections			cost	
Acinetobacter baumannii	Ventilator-associated pneumonia, bloodstream infections	Carbapenemase production; efflux pumps; outer membrane protein loss	Multidrug-resistant *A. baumannii* (MDRAB)	Common in paediatric ICUs; poor prognosis in severe infections	WHO, 2024
***Salmonella enterica* (non-typhoidal)**	Gastroenteritis, invasive bacteremia	Plasmid-mediated multidrug resistance; fluoroquinolone resistance (gyrA mutation)	MDR *Salmonella*; ciprofloxacin-resistant strains	Complicates treatment of enteric fever and sepsis; increased hospitalization	CDC, (2023); WHO, (2024)
Neisseria meningitidis	Meningitis, septicemia	β-lactamase production; penA gene mutations	Penicillin- and ciprofloxacin-resistant *N. meningitidis*	Reduced effectiveness of prophylaxis and empiric therapy	(WHO, 2024)
Enterococcus faecium	Nosocomial infections, bloodstream infections	vanA and vanB genes conferring vancomycin resistance	Vancomycin-resistant *Enterococcus* (VRE)	Difficult-to-treat infections; limited options like linezolid or daptomycin	CDC, (2023); WHO, (2024)

ESBL: Extended-Spectrum β-Lactamase; CRE: Carbapenem-Resistant Enterobacterales; NDM/KPC/OXA-48: Common carbapenemase enzymes; MRSA: Methicillin-Resistant Staphylococcus aureus; VRE: Vancomycin-Resistant Enterococcus

1.2 Justification of Project

Globally, the estimated population of children below 18 years is 2.4 billion, while 27% of this population is estimated to be younger than 5 years (Browne *et al*., 2021). Due to a combination of environmental and behavioural variables, facilitated by behaviours including playing in polluted areas, not washing your hands, consuming contaminated food and drink, and interacting closely with people in crowded places like daycare centres and schools (Efunshile *et al*., 2016; Ezeonu *et al*., 2020). Common paediatric infections brought on by these exposures include skin infections, respiratory tract infections, and diarrheal illnesses, all of which are commonly empirically treated with antibiotics (Okomo *et al*., 2019). In 2019, drug-resistant illnesses accounted for the death of at least 1.27 million children globally, while in 2021, an estimated 3.68 million children died from resistant infections, perhaps contributing to their deaths (Naghavi, 2022).

Children are major consumers of antimicrobial agents and have high rates of AMR. Children underdeveloped immune systems make them more susceptible to infectious diseases such as pneumonia and meningitis and are treated with antibiotics. Insufficient understanding of the resistance mechanisms of common paediatric pathogens and lack of paediatric-specific data have both contributed to the overuse and misuse of antibiotics, making antibiotic resistance in paediatric infections a growing threat to public health. Macrolide- and clindamycin-resistant *Streptococcus pneumoniae* and *Bordetella pertussis* are serious problems for children in some countries, such as China. The detection rate of carbapenem-resistant *Enterobacteriaceae* was also higher in children than in adults. Because children are in a special period of growth and development, their pharmacokinetic (PK) and pharmacodynamic (PD) characteristics vary widely, making it difficult to determine age-dependent doses. The lack of paediatric-specific data is also an important cause of the irrational use of antibiotics in

children, leading to treatment failure and antibiotic resistance. Similarly, a retrospective study conducted in a Lagos Children's hospital found that over half (52.0%) of cultures from admitted children yielded bacterial growth, with neonates exhibiting the highest positivity rate. The most frequently isolated pathogens are *Staphylococcus aureus* (32.1%), *Escherichia coli* (28.2%), and *Klebsiella* spp. (20.5%) demonstrated substantial resistance, particularly to first-line antibiotics such as ampicillin (58 resistant isolates) and amoxicillin–clavulanic acid (54 resistant isolates).

These organisms are notorious for causing a range of infections that contribute significantly to paediatric morbidity and mortality. In neonatal intensive care units (NICUs) and paediatric wards, *Klebsiella* infections are frequently reported, with emerging antibiotic resistance compounding their clinical impact. *Klebsiella* spp. are responsible for a variety of serious infections in children, particularly among neonates and hospitalized patients. One of the most severe conditions caused by these bacteria is neonatal sepsis, which remains a leading cause of death in NICUs across Nigeria. This systemic infection often arises in the first few days of life and requires urgent medical intervention (Onwuezobe *et al*., 2015). In addition to sepsis, pneumonia caused by *Klebsiella spp*. is prevalent, especially among ventilated or immunocompromised children. The bacteria can also cause urinary tract infections (UTIs), meningitis, and liver abscesses, reflecting its ability to affect multiple organ systems. These infections are particularly dangerous in healthcare settings where infection control practices are inadequate (Ogbera *et al*., 2011). The symptoms associated with *Klebsiella* infections vary depending on the type and severity of the illness. In cases of neonatal sepsis, affected infants typically present with fever, lethargy, and poor feeding (Onwuezobe, 2015; Ogbera, 2011). The clinical symptoms of *S. aureus* infections vary depending on the site and severity of the infection. Children with skin infections often present with fever and boils or pustules, which may be painful and filled with pus. When the

lungs are involved, symptoms may include cough, difficulty breathing, and chest pain, especially in pneumonia cases (Okonko *et al*., 2019).

Monitoring AMR across diverse populations not only aids in guiding the empirical antibiotic use in clinical practice, but also facilitates the development of intervention and prevention strategies, however, few studies have evaluated AMR in children within the geographical confine of Edo state, these limited knowledge could be associated with the prevalence of AMR, and also the limited understanding of the resistance mechanisms associated with common paediatric pathogens, coupled with a lack of paediatric-specific data, has led to both overuse and misuse of antibiotics, thereby exacerbating antibiotic resistance among children posing a significant concerns to stakeholders.

The high burden of AMR among Nigerian children highlights a critical public health challenge, particularly in resource-limited settings like Edo State. These symptoms imposed by the resistance pathogens may range from mild skin conditions to life-threatening systemic illnesses. Early recognition of symptoms, proper hygiene practices, and access to effective antimicrobial therapy are essential to controlling these infections. Strengthening hospital infection control and surveillance systems can further reduce morbidity and mortality associated with AMR in the child population. Thus, this study aims to assess AMR in Children.

1.3 Significance of the Study

This study holds considerable academic, clinical, and public health relevance in the ongoing fight against antimicrobial resistance (AMR). From an academic perspective, the research will contribute to the growing body of literature on bacterial resistance patterns in paediatric populations, providing locally relevant data to fill existing knowledge gaps in sub-Saharan Africa. The findings will enhance scientific understanding of the mechanisms and epidemiological

trends of antimicrobial resistance, supporting comparative studies across regions and informing future research on pathogen genomics, resistance determinants, and antimicrobial stewardship interventions (World Health Organization, 2024).

Clinically, the study's findings will provide essential baseline data to guide empirical therapy and infection prevention strategies, particularly in paediatric healthcare settings where evidence-based antibiotic selection is critical to improving patient outcomes. By identifying resistance profiles of prevalent pathogens, healthcare providers can make more informed choices regarding antibiotic prescription, reducing the risk of therapeutic failure and minimizing unnecessary use of broad-spectrum antimicrobials (Federal Ministries of Agriculture, Environment, Health and Social Welfare, Federal Republic of Nigeria, 2024). These results can further support hospital antibiotic stewardship programs and inform the development of local antibiograms to strengthen diagnostic and treatment protocols.

From a public health standpoint, the study aligns with the One Health approach, recognising the interconnection between human, animal, and environmental health in AMR transmission and control (CDC, 2023). The data generated will support national AMR surveillance frameworks, such as Nigeria's participation in the Global Antimicrobial Resistance and Use Surveillance System (GLASS), and will inform policy decisions related to infection prevention, regulation of antibiotic use, and capacity building in laboratory diagnostics. Ultimately, the study's findings will contribute to the implementation and evaluation of Nigeria's National Action Plan on AMR (2024–2028), helping to strengthen coordinated responses across the healthcare, agricultural, and environmental sectors (WHO Regional Office for Africa, 2024).

1.4 Aim and objectives

1.4.1 Aim

This study aims to comprehensively investigate the antimicrobial susceptibility patterns of *Staphylococcus aureus* isolated from paediatric patients.

1.4.2 Objectives

The specific objectives of the study are to:

1. Identify and characterize *Staphylococcus aureus* isolates obtained from paediatric clinical samples.
2. Determine the antimicrobial susceptibility patterns of the *S. aureus* isolates to commonly used antibiotics.
3. Assess the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among the isolates.
4. Identify possible factors contributing to antimicrobial resistance in paediatric infections.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and Definition of *Staphylococcus aureus*

Staphylococcus aureus was named after the Greek word *Staphyle* ("a bunch of grapes") and *Kokkos* ('grain of berry'), referring to its grape-like cluster morphology. *S. aureus* is a Gram-positive, catalase- and coagulase-producing coccus (Khan, 2017). Clusters of *S. aureus on* culture medium characteristically appear as a golden colour, as a result of the carotenoid pigments, and can tolerate high salt concentrations (Taylor and Unakal, 2023).

The bacterium belongs to the genus *Staphylococcus*, family Staphylococcaceae, order Bacillales, and phylum Bacillota (Sayers, 2019). Members of this group are spherical (cocci) and divide in multiple planes to form clusters. *Staphylococcus* differs from *Streptococcus* based on its production of catalase and its arrangement. They are approximately 0.5-1.5 μm in diameter, non-motile, non-spore-forming, facultative anaerobes that usually form in clusters.

Staphylococcus aureus is uniquely resistant to adverse conditions such as high salt content and osmotic stress (Fayisa, 2023). *S. aureus* causes significant disease to both humans and animals including illness such as mastitis; mammary gland infection in cows, lameness in poultry, urinary tract infection (UTI), paediatric osteomyelitis, paediatric serum sickness, leptospirosis, impetigo, bacteraemia, bacterial endocarditis, minor skin and soft tissue infections resulting to severe life-threatening bloodstream infection (Rao et al., 2023; Khatija et al., 2023; Rock, 2020). Both community-associated and hospital-acquired infections with Staphylococcus aureus have increased in the past 20 years. *S. aureus* is found in the environment and forms part of the normal flora of the skin, nasal passages, and mucous membranes of healthy individuals. While often harmless on healthy skin*, S. aureus* can cause serious infections when it enters the bloodstream

or internal tissues, leading to boils, abscesses, and other severe conditions (Becker et al., 2014; Rock, 2020).

S. aureus has various strains that harbour the mecA gene, which is associated with MRSA, one of the most common causes of healthcare and community-associated infection (Lakhundi and Zhang, 2018; David and Daum, 2020). The mecA gene is located on the staphylococcal cassette chromosome mec (SCCmec), a mobile genetic element. It encodes penicillin-binding protein 2a (PBP2a), which enables cell wall synthesis in the presence of β-lactam antibiotics (Siddiqui and Koirala, 2022). SCCmec elements are categorized into types I–VI based on their genetic composition (IWG, 2009). Types I–III are typically associated with healthcare-associated MRSA and carry multiple resistance genes, whereas types IV–VI are linked to community-associated MRSA and primarily harbour the mecA gene (David and Daum, 2020).

The history of *Staphylococcus aureus* spans from ancient times. Although *S. aureus* existed long before its discovery, it was first identified as a bacterial pathogen in human wound infections by Ogston in 1880 and later described by Rosenbach in 1884 (Licitra, 2013). Ogston observed grape-like clusters of cocci in pus, and Rosenbach distinguished the golden-coloured strains as *S. aureus*. For decades prior to antibiotics, *S. aureus* infections had very high mortality.

Alexander Fleming accidentally discovered penicillin in 1928 from the mould Penicillium notatum, which contaminated a culture of S. aureus (Fleming, 1929). The pure penicillin compound from the mould was successfully extracted in 1939 by Howard Walter Florey and Ernst Boris Chain (Chain et al., 1940). Penicillin was first used clinically in 1941 and initially reserved for soldiers during World War II (Fletcher, 1984; Abraham et al., 1940). The drug was proven so effective in the treatment of several bacterial infections that in a short time it earned

the reputation as a ‘magical bullet’ or a ‘miracle drug’ Straand, 2008). Penicillin introduced a cure, but widespread penicillin-resistant strains emerged by the late 1940s.

2.1.1 The Emergence and Spread of Resistant *Staphylococcus aureus*

Long before antibiotics were conceptualized, *Staphylococcus aureus* had already established itself as a common colonizer and opportunistic pathogen (de Lencastre et al., 2017). The introduction of penicillin in the early 1940s dramatically reduced mortality from staphylococcal infections, but within just a few years, resistant strains appeared (Turner et al., 2007; Chambers and DeLeo, 2020). By 1945, approximately 80% of S. aureus clinical isolates produced plasmid-encoded β-lactamase (penicillinase), rendering penicillin ineffective (Turner et al., 2007; Chambers and DeLeo, 2009). That enzymatic mechanism became globally widespread, and penicillin resistance remains present in nearly all clinical strains today (Turner et al., 2007; Chambers and DeLeo, 2020; Rio et al., 2008).

A dominant clone, phage type 80/81, was responsible for a 1950s pandemic of skin, sepsis, and pneumonia infections across multiple continents (Turner et al., 2020). Hospital-based outbreaks gradually spilled into community settings before declining after methicillin was introduced (Turner et al., 2020; Rio et al., 2008).

Methicillin, first used in 1959, was engineered to resist β-lactamase hydrolysis (de Lencastre et al., 2017). Yet by 1961, within two years, clinical MRSA (methicillin-resistant *S. aureus*) isolates emerged in the UK, carrying the mecA gene, which encodes penicillin-binding protein 2a (PBP2a) with low affinity to beta-lactams (Chambers and DeLeo, 2020; Turner et al., 2017). This novel mechanism conferred broad resistance across all β-lactam classes (Chambers and DeLeo, 2020; Turner et al., 2017).

Genomic studies further revealed that many MRSA lineages acquired mecA-bearing SCCmec elements even before widespread methicillin use selected by earlier exposure to penicillin (Monegro et al., 2017; de Lencastre et al., 2017). The first epidemic MRSA clone, ST250-MRSA-I, likely evolved in the mid-1940s and spread throughout Europe in the 1960s (de Lencastre et al., 2017; Monegro et al., 2017).

During the 1970s and 1980s, newer SCCmec types (II, III) appeared in MRSA strains that became endemic in hospitals worldwide (Turner et al., 2007). For example, in the US, MRSA prevalence rose from ~2% in 1975 to nearly 30% by 1991; by 2003, over 50% of ICU isolates were MRSA (Turner et al., 2017; de Lencastre et al., 2007; Chambers and DeLeo, 2020).

Extensive MRSA infections prompted increased use of vancomycin, long considered the drug of last resort. However, by the early 2000s, vancomycin-intermediate (VISA) and even vancomycin-resistant (VRSA) strains began to emerge. These strains acquired vanA genes from Enterococcus, limiting therapeutic options severely (Khoshnood et al., 2017; CDC, 2003).

By the late 1990s, MRSA had transcended hospitals; community-associated MRSA (CA-MRSA), harboring smaller SCCmec IV elements and toxins like PVL, began infecting otherwise healthy individuals (Chambers and DeLeo, 2020; de Lencastre et al., 2017). Livestock-associated MRSA (LA-MRSA), especially the ST398 lineage, emerged in pig farms in the early 2000s and then spread to humans, driven by agricultural antibiotic use (Wired, 2012; Khoshnood et al., 2017).

2.1.2 *Staphylococcus aureus* Evolutionary Mechanisms of Adaptation

At the molecular level, resistance emerged via two primary routes: enzymatic antibiotic degradation (e.g., β-lactamase for penicillin) and target alteration mechanisms (e.g., PBP2a produced by mecA), which prevent antibiotic binding (Chambers and DeLeo, 2020; de Lencastre et al., 2017). Mobile genetic elements like plasmids, SCCmec, and transposons have facilitated

horizontal transfer of resistance genes across strains and species (de Lencastre et al., 2020; Turner et al., 2017; Khoshnood et al., 2017).

Selective pressures from clinical and agricultural antibiotic overuse accelerated these adaptations. For example, longitudinal genomic sequencing in a patient over twelve weeks revealed over 30 mutations evolving under treatment, demonstrating rapid in vivo selection (Wired, 2007).

Resistance beyond β-lactams emerged through mutations in antibiotic targets (e.g., DNA gyrase, topoisomerase IV) and via efflux pumps (e.g., NorA), conferring resistance to fluoroquinolones, macrolides, tetracyclines, and more (MDPI, 2023; Chambers and DeLeo, 2009).

In some cases, linezolid resistance (oxazolidinone class) appeared as early as 2001, mediated by point mutations or acquisition of cfr genes. A notable outbreak in Spain involved 12 ICU patients infected with linezolid-resistant *S. aureus* carrying cfr, with 50% mortality; reducing linezolid use helped control the epidemic (Wired, 2010).

Enzymatic inactivation involves the production of enzymes that chemically modify or degrade antibiotics. The most prominent example is β-lactamases, which hydrolyse the β-lactam ring in penicillins, cephalosporins, and carbapenems, thereby disrupting the ability of these antibiotics to inhibit the synthesis of the microorganisms' cell walls (Bush and Bradford, 2016). There are more than 1,000 known β-lactamases, including CTX-M and other extended-spectrum β-lactamases (ESBLs) and narrow-spectrum enzymes. Common in carbapenemases such as NDM-1 and KPC, as well as *Escherichia coli* and *Klebsiella pneumoniae* (Bush and Bradford, 2016). Since its discovery in 2008, NDM-1 has proliferated around the world, causing resistance to carbapenems of last resort and making the management of Gram-negative infections more difficult (Walsh *et al*., 2011). By adding chemical groups that prevent antibiotic action, other

enzymes, like aminoglycoside-modifying acetyltransferases, and enzymes, like chloramphenicol acetyltransferases, inactivate aminoglycosides and chloramphenicol, respectively (Munita and Arias, 2016). Zhang *et al*. (2024) in their study conducted to evaluate antimicrobial resistance in clinically ill paediatric patients, found that gram-negative bacteria exhibit a broader spectrum of resistance, predominantly resistant to cephalosporins, β-lactams/β-Lactamase inhibitors, carbapenems and sulfonamides, while the prevalent pathogens showed variable resistance to different class antimicrobial; *K. pneumoniae* has considerable resistance to cephalosporins, β-lactams/β-Lactamase inhibitors, and sulfonamides, with rates of 46.15%, 39.29%, and 44.44%, respectively. Resistance to carbapenems is significantly lower, at 21.05%. *P. aeruginosa* exhibited increased resistance to carbapenems (33.33%), but lesser resistance to cephalosporins, β-lactams/β-Lactamase inhibitors, and sulfonamides (0%, 5.26%, and 0%). *A. baumannii* is highly resistant to cephalosporins (91.67%), carbapenems (100%), and sulfonamides (100%) 66.67% resistance to β-lactams and β-lactamase inhibitors. Gram-positive bacteria showed resistance to antibiotics such as penicillins, macrolides, and lincosamides. Levofloxacin was among the antibiotics with sample counts surpassing 22 (50%) in the AST analysis.

Resistance in bacteria to some antibiotics can either be intrinsic or acquired through gene mutations or via HGT. Bacterial species with intrinsic resistance can survive the actions of certain antibiotics due to innate structural or functional features (Zhang *et al.,* 2024). Intrinsic resistance is achieved via inherent structural or functional characteristics of the molecular target. The antibiotic daptomycin is active against Gram-positive bacteria but ineffective against Gram-negative bacteria; this is the result of innate differences in the cytoplasmic membrane composition between the Gram-positives and Gram-negatives. The Gram-negative cell membrane comprises of less anionic phospholipids than the cytoplasmic membrane of Gram-

positive bacteria (Blair *et al*., 2014). This reduction in the fraction of anionic phospholipids affects the $Ca2^{+}$-dependent insertion efficiency of daptomycin into the cytoplasmic membrane, which is needed for its antibacterial activity.

Recent studies have determined that several genes are responsible for the innate resistance that exists towards certain classes of antibiotics, such as fluoroquinolones, β-lactams, and aminoglycosides. The key resistance genes are thioredoxin reductase (trxB), thioredoxin A (Trx A), SapC, DacA, FabI, and D-Ala-D-Ala carboxypeptidase. So, combined antagonism of these gene products could help to boost the activity of existing drugs such as rifampin, aminoglycosides, as well as some β-lactams in the treatment of infection (Liu *et al*., 2019). The genes responsible for innate resistance were identified from high-density genome mutant libraries and high-throughput screening studies. To create the libraries, either mutagenesis by random transposon insertion or targeted insertion was carried out in bacteria such as *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* (Liu *et al*, 2019). This library screening approach is particularly effective in discovering possible novel drug combinations that will enable inhibition of intrinsic resistance mechanisms (Blair *et al*., 2014). Consequently, the spectrum of activity of other antibiotics can be extended to other pathogens beyond their common target species.

In addition to intrinsic resistance, there are several other resistant mechanisms that can be acquired by bacteria, all of which fall into three primary groups: prevention of access to target; mutational changes in antibiotic targets and finally, modification of targets (Blair *et al*., 2014).

To lessen or eliminate drug binding, bacteria modify the target sites of antibiotics. The mecA gene in MRSA produces PBP2a, an alternative penicillin-binding protein with a low affinity for β-lactam antibiotics, which permits the continuation of cell wall construction (Munita and Arias,

2016). Similarly, mutations in the rpoB gene, which codes for the RNA polymerase β-subunit and modifies the rifampicin-binding site, cause rifampicin resistance in *Mycobacterium tuberculosis* (Telenti *et al*., 2023). Tet(M) and other ribosomal protection proteins, which stop tetracycline from attaching to bacterial ribosomes and disrupting protein synthesis, are frequently involved in tetracycline resistance (Munita and Arias, 2016).

Denial of access to an antibiotic to its target can be exemplified by the resistance of Enterobacteriaceae to carbapenems, which is due to reduced permeability of the bacterial membrane. Hydrophilic antibiotics diffuse into the bacterial cell via the porin proteins in the outer membrane. OmpC and OmpF of E. coli are typical examples of the major porins found in most Enterobacteriaceae. Several studies have suggested that resistance strategies employed by bacteria include the downregulation of expression of porin proteins or the substitution of major porins with more-selective membrane channels. So, the resistance to carbapenems in the Enterobacteriaceae in the clinical setting persists even without carbapenemase production by the bacteria; instead, critical mutations reduce porin production or result in the expression of mutant porin alleles (Wozniak and Waldor, 2018). The prevention of access to the antibiotic's target can be achieved through increased efflux of the antibiotic. The efflux pump of bacteria transports antibiotics actively out of the cell in bulk. This contributes significantly to the intrinsic resistance in Gram-negative bacteria to a variety of drugs that are used in the treatment of bacterial infections. The five major types of efflux pumps that have been identified include the ATP-binding cassette family (ABC), the multidrug and toxic compound extrusion family (MATE), the major facilitator superfamily (MFS), the small multidrug resistance family (SMR) and the resistance-nodulation-cell-division family (RND) (Munita *et al.,* 2016). Multidrug-resistant (MDR) efflux pumps transport a vast range of structurally different substrates. There are

several genes encoding the MDR efflux pumps located on bacterial chromosomes, however, some of those genes can be transferred between bacteria, due to mobilization on the genes onto plasmids (Quinn *et al*., 2022). Recently, it has been found out that the IncH1 plasmid, isolated from Citrobacter freun-dii, has a gene cassette encoding a novel resistance modulation division (RND) pump together with the gene for New Delhi metallo-β-lactamase 1 (NDM1) (Piddock *et al.,* 2016). This development is a major concern since this indicates that these specific resistances can be transmitted between bacteria on plasmids, which may facilitate the spread of novel resistances to other bacterial pathogens that are clinically relevant.

2.3 Biology and Structure of *Staphylococcus aureus*

S. aureus is a facultative anaerobe with a thick peptidoglycan-rich cell wall and teichoic acids. It grows in grape-like clusters (tetrads) due to its division in multiple planes (Taylor and Unakal, 2023). The pathogenic prowess of *S. aureus* is largely due to its broad virulence factors, which allow it to cling to host tissues, elude immune responses, and harm host cells (Cheung et al., 2021). Surface proteins including clumping factors (ClfA, ClfB) and fibronectin-binding proteins aid in attachment to host cells and tissues. Secreted toxins such as α-hemolysin, PVL, and TSST-1 may cause tissue damage and systemic toxicity (Linz et al., 2023). Furthermore, *S. aureus* readily forms biofilms on devices, aiding the occurrence of persistent chronic infection by protecting bacteria from antimicrobial therapy and human defence systems. These factors also facilitate their colonization (especially of nose/skin) and invasion under favourable conditions (Touaitia et al., 2025).

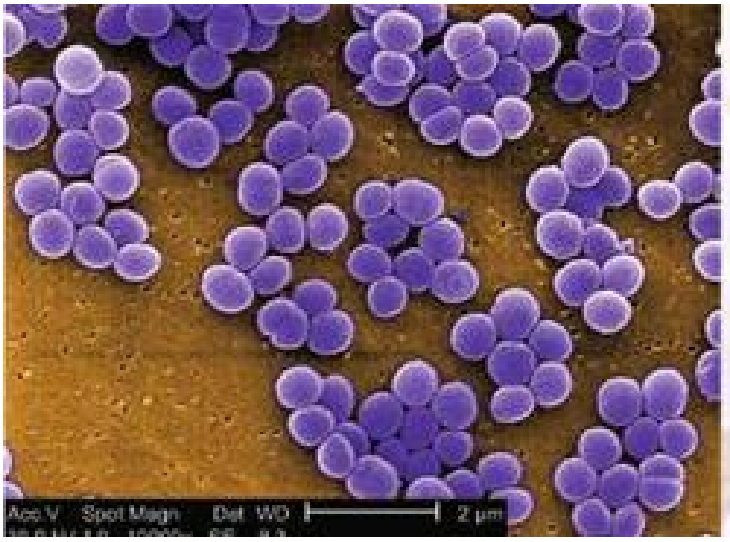

Figure 2.1: Typical cocci shape of *Staphylococcus aureus* in clusters

(Prescott, 2018)

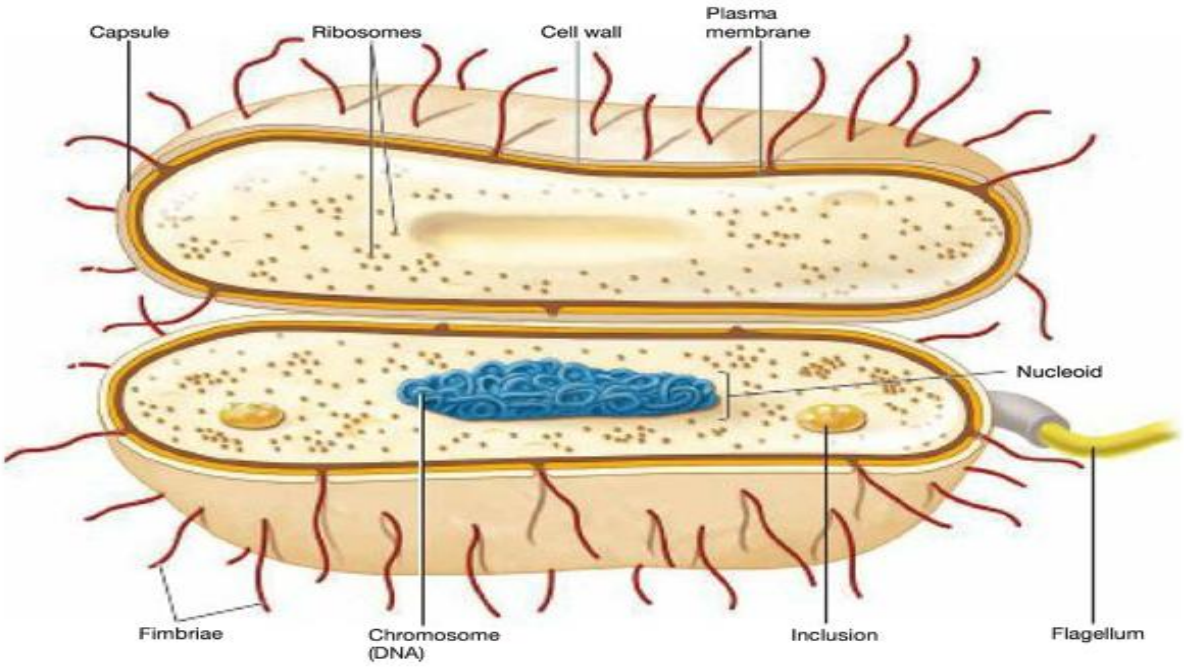

Figure 2.2: Typical structure of gram-positive bacterial

2.4 Infections Caused by *Staphylococcus aureus*

In children, *S. aureus* can cause a broad spectrum of illness. It is a common cause of skin and soft tissue infections (impetigo, abscesses, furuncles, cellulitis, scalded skin syndrome) (Taylor and Unaika, 2023). It also causes invasive disease, *S. aureus* bacteraemia can lead to osteomyelitis, septic arthritis, pneumonia (often post-viral), endocarditis, and device-related sepsis (Taylor and Unaika, 2023; Touita et al., 2025). Toxin-mediated syndromes such as toxic shock and food poisoning are well-known. In neonates, *S. aureus*, including MRSA is a leading cause of sepsis; for example, one Nigerian study found *S. aureus* in 56.4% of neonatal sepsis cases, and 56.3% of those staphylococcal cases were methicillin-resistant (Egbuobi et al., 2014). Overall, *S. aureus* is among the top paediatric pathogens globally due to its high virulence and capacity for resistance.

2.4.1 Urinary Tract Infection (UTI)

In children, urinary tract infection (UTI) is the most prevalent bacterial infection within the first seven years of life, affecting 8% and 2% of girls and boys, respectively (Kanellopoulos et al., 2016). *Staphylococcus aureus* is an uncommon but clinically significant pathogen in paediatric urinary tract infections (UTIs). Unlike the more frequent Gram-negative uropathogens, *S. aureus* UTIs often arise from hematogenous seeding, urinary catheters, or anatomical abnormalities, and carry a higher risk of systemic complications (Kanellopoulos et al., 2016).

Abnormalities of urinary tract abnormalities, like congenital, can cause a high risk of UTI in some children (Isac et al., 2021). In 30% of children with CAKUT (congenital anomalies of kidney and urinary tract) are at danger for the development of UTI in children. Unidirectional flow of urine changes due to vesico-ureteral reflux (VUR) (Ludwikowski and Gonzalez, 2019). While pyelo-ureteral junction obstruction (PUJO) leads to stasis, in which both increase the risk

of multiplying pathogenic microorganisms (Harper et al., 2022). At the age of 1 month and 11 years, more than 8% of children will experience at least one UTI, and during the first six to 12 months after an initial UTI, more than 30% of kids and newborns experience repetitive infections (Buosenso et al., 2022). The most common aetiology of UTIs is due to more than 95% of bacteria. *Escherichia coli (E. coli)* is the most frequent causative organism of UTIs and is responsible for more than 80% (Ouner et al., 2021). Staphylococcus aureus is also responsible for the anomalies in the urinary tract of children, especially in immune compromised children (Ouner et al., 2021).

Only a proper identification of the local pathogen and information on the susceptibility patterns and any related risk factors can provide appropriate treatment for UTIs (Ouner et al., 2021). Because of incorrect antibiotic use, the bacterial sensitivity pattern of common pathogens is gradually changing in all countries (Ponvelil et al., 2020). To decrease the morbidity rate of UTIs, proper treatment is required. The non-specific signs and symptoms of UTIs in children under the age of two years can make it challenging to diagnose UTIs (Aslam and Aslam, 2022). Children with simple UTIs may respond to sulphonamides, amoxicillin, trimethoprim-sulfamethoxazole, or cephalosporins, with amoxicillin, sulphonamides, trimethoprim-sulfamethoxazole, or cephalosporins concentrating in the lower urinary tract (Wang et al., 2021).

2.4.2 Skin and Soft Tissue Infection caused by *Staphylococcus aureus*

Skin and soft tissue infections (SSTIs) are common bacterial infections associated with significant morbidity and with admission in ambulatory settings, including the emergency department (Bassetti et al., 2014; Rajan, 2012). The most common cause of these infections is *Staphylococcus aureus* which colonizes the skin, mouth, and upper respiratory system (Yakut et al., 2024). Although methicillin-resistant *S. aureus* (MRSA) strains are a main concern for

clinicians, methicillin-susceptible *S. aureus* (MSSA) is also very important as they are the most common causative agents of SSTIs in many parts of the world (Watanabe et al. 2020). Management of SSTIs has become more challenging with the emergence of resistance to commonly used antibiotics (Tolan et al., 2018). The epidemiology of skin and soft tissue infections (SSTIs) caused by *S. aureus*, as well as the antimicrobial susceptibility *of S. aureus*, varies based on the patient population and geographic regions (Poulakou et al., 2019).

2.4.3 Asymptomatic infection of the Nasopharynx in Children

The nasopharynx and anterior nares are considered the major habitats of *S. aureus* with nasal transmission observed from 16.8% to 90% of strains (Askarin et al., 2009; Hema-Ouangraoua, 2021). When *S. aureus* is acquired as an asymptomatic nasal carriage, it can gradually turn into a persistent carriage where children are especially susceptible to infection due to the systemic invasion of the pathogen (Askarian et al., 2009) According to a study conducted by Esposito et al. (2024) out of the 497 healthy children, 264 (53.1%) were carriers of *S. aureus*, with 195 (39.2%) being nasal carriers. However, the study revealed that only three nasal samples (0.6%) were methicillin-resistant *S. aureus* (MRSA), indicating that the prevalence of MRSA nasal carriage among healthy individuals is relatively low. There are few studies on the prevalence of *S. aureus* in children. Studies in Nepal and Vietnam found *S. aureus* in the upper respiratory tract in 15% and 29.8% of children, respectively (Van Nguyen et al., 2014). Over the years, *S. aureus* has developed resistance against penicillin and methicillin, which were once effective treatments for the bacteria in the mid-20th century (Guo et al., 2020). Despite advancements in antibiotic therapy, MRSA still poses a significant risk to global health. Reports from different studies showed that the MRSA isolates were resistant to ciprofloxacin (88.9%) and erythromycin (72.2%) among the identified cases. It was also found that about 20 to 100% of *S. aureus* isolates were susceptible to different antibiotics (Taz et al., 2019).

2.5 Diagnosis of *Staphylococcus aureus* infections in Children

Prompt and accurate diagnosis of *S. aureus* infections in children is critical for effective management. However, the presence of *S. aureus* in culture is normally insignificant since this bacterium is normally present on the skin, nose and pharynx of many humans and animals. The organism is readily cultured from nasopharynx or skin, or by culture of suspicious lesions (Harris et al., 2022).

Clinical diagnosis relies on the culture of clinical specimens (blood, pus, sputum, etc.). *S. aureus* grows on routine media and is identified by morphology (golden colonies), Gram stain (Gram-positive cocci in clusters), and biochemical tests (catalase-positive, coagulase-positive) (Taylor and Unakal, 2023). Rapid molecular methods (e.g. PCR for *mecA*, 16S rRNA) may be used in specialized labs. In practice, routine culture "reveals the diagnosis" in most cases (Rasigade et al., 2014). Once grown, isolates undergo antimicrobial susceptibility testing (e.g. disk diffusion or automated systems) to guide therapy. Notably, *S. aureus* is a common nasal commensal, so culture from non-sterile sites must be interpreted with clinical context. Laboratory protocols often include cefoxitin/oxacillin screening to identify MRSA strains (Lowy, 2023).

S. aureus infections often initially present as skin infections, characterized by painful, red, swollen bumps that may resemble spider bites, pimples, or boils, sometimes accompanied by fluid leakage (Whittington et al., 2025). These lesions may be warm to the touch, grow rapidly, or fail to heal. If the infection spreads systemically, symptoms can escalate to include fever, chills, severe headache, sleepiness, dizziness, or fainting, necessitating immediate medical attention (Whittington et al., 2025).

The challenges in diagnosing and treating *S. aureus* infections in children, often presenting as non-specific skin lesions, emphasize the need for heightened clinical suspicion and timely laboratory confirmation to prevent systemic spread and severe outcomes. Many *S. aureus* infections, particularly MRSA, begin as simple skin cuts or scrapes, if left untreated, these localized infections can quickly progress to damage nearby tissue, infect other individuals, and disseminate throughout the body, leading to life-threatening conditions such as blood poisoning, pneumonia, or shock (O'Hanlon et al., 2019). This progression underscores the importance of early recognition and intervention.

Diagnosis typically involves a physical examination and laboratory tests, including skin swabs, cultures of samples from blood, sputum, or fluid from a sore to identify *S. aureus* and determine its susceptibility (Cardenas et al., 2020) Imaging tests may be performed if the infection is suspected to have spread to joints or bones (Duguid et al., 2021). The advent of rapid diagnostic techniques, such as polymerase chain reaction (PCR) tests for *S. aureus* bacteraemia (rPCR), including *mecA* gene detection, significantly expedites antibiotic optimization. These tests can differentiate *S. aureus* (including MRSA) within hours of positive blood culture growth, allowing for earlier, more effective antimicrobial therapy (Whittington, 2025).

2.6 Epidemiology of *Staphylococcus aureus* in Paediatric Populations

Staphylococcus aureus is a common commensal, colonizing approximately 30% of the human population, often asymptomatically in the nasal passages (Tong et al., 2015). In young children, the global pooled prevalence of asymptomatic *S. aureus* colonization was found to be 25.1% (95% CI 21.4 to 28.8) among a cohort of 21,416 individuals (Al-lede et al., 2024). Focusing on Methicillin-Resistant

Regarding the global prevalence of *S. aureus* (MRSA), the overall pooled estimated global prevalence of MRSA colonization in the paediatric population, derived from 124 studies encompassing 44 million participants, was 5% (95% CI 4–5%).6 For asymptomatic MRSA colonization specifically in young children, the pooled global prevalence was slightly lower at 3.4% (95% CI 2.8 to 4.1) (Al-lede et al., 2024)

When considering active infections, MRSA accounted for 28.74% of S. aureus isolates in paediatric bone and joint infections.8 In other analyses, MRSA was identified in 35.3% of blood infections and 27.3% of respiratory cultures (Simões and Maresca, 2025) The incidence of *S. aureus* bacteremia (SAB) exhibits a distinct age-dependent pattern, with high rates observed in the first year of life, a subsequent low incidence throughout young adulthood, and a gradual increase with advancing age (Tong et al., 2015). Furthermore, the incidence of infective endocarditis (IE) increased from 11.4 per 100,000 person-years in 1999 to 16.6 per 100,000 person-years in 2006, with the majority of this rise attributable to *S. aureus* (Tong et al., 2015).

The prevalence of MRSA colonization in paediatric populations demonstrates significant geographical variability. It is highest in Asia, with an estimated 8%, and lowest in Europe, at 3%.6 similarly, for asymptomatic MRSA colonization in young children, higher prevalence rates were reported in Asia (5.8%) and South America (5.6%) compared to Europe (0.6%) (Al-lede et

al., 2024) A concerning temporal trend indicates that the overall asymptomatic MRSA colonization prevalence among young children has increased over the last 10–15 years, rising from 2.8% (based on 2000-2010 data) to 3.4%.

This rise suggests that current control measures or public health messaging are insufficient to curb colonization rates in this vulnerable group. If colonization is increasing, it implies that environmental factors (e.g., contaminated surfaces in daycare, sports equipment) or behavioural factors (e.g., hand hygiene, sharing personal items) that facilitate transmission remain highly active or are worsening (Stanford Medicine, 2025). This trend underscores the need for more effective, targeted prevention strategies that extend beyond antibiotic stewardship, focusing on hygiene and decolonization, particularly in settings where children congregate (Al-lede et al., 2024).

A notable "tenfold increase in the incidence of MRSA infection among children in the USA between 1999" and 2008 has been documented.6 Additionally, the incidence of *S. aureus* infection among hospitalized children in the United States significantly rose from 20.8 cases per 1000 admissions in 2002 to 35.8 cases per 1000 admissions in 2007, a surge driven exclusively by MRSA (Gerber et al., 2019). Community-associated MRSA (CA-MRSA) has emerged as a primary driver behind the increase in nosocomial MRSA infections The prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) infection in Nigeria varies across studies, ranging from 2.3% to 47.4% among clinical isolates. Recent studies indicate an increase in MRSA prevalence, with some showing a 2.3-fold rise between 2009 and 2013. A high prevalence of resistance to other antibiotics, such as macrolides, tetracyclines, quinolones, and trimethoprim-sulfamethoxazole, has also been reported among Staphylococcus aureus isolates, including those susceptible to methicillin (MSSA) (Abubakar and Sulaiman, 2018).

This indicates that *S. aureus*, particularly MRSA, is no longer solely a hospital-acquired threat but has become a pervasive community pathogen. Consequently, traditional hospital-centric infection control measures are insufficient. Prevention efforts must now extend to community settings such as schools, sports facilities, and daycare centers, emphasizing general hygiene, proper wound care, and increased awareness among parents and children. The higher prevalence in young children and specific risk factors associated with group activities further necessitate community-wide public health campaigns and interventions (Al-lade, 2023).

Globally asymptomatic colonization of children is common worldwide. A recent meta-analysis found that about 25.1% (95% CI 16.2–36.0%) of young children globally carry *S. aureus* in the upper respiratory tract, while about 3.4% (1.8–5.7%) carry MRSA. This underscores that most children are colonized by methicillin-susceptible *S. aureus* (MSSA). However, MRSA colonization appears to have increased globally in recent years, likely driven by both healthcare- and community-associated strains. *S. aureus* infections (both MSSA and MRSA) occur worldwide; incidence varies by region and healthcare setting.

Regional data from studies conducted in Africa shows that MRSA carriage and infection are significant. A 2025 African meta-analysis reported pooled MRSA carriage of 4.7% (95% CI 3.0–6.8%) among children and 4.1% (2.6–6.3%) among healthy community members in Africa, In their studies they noted that healthcare workers (13.6%) and hospitalized patients (12.9%) had much higher MRSA rates (Azzam et al., 2025). In sub-Saharan settings, *S. aureus* remains endemic, complicated by rising antibiotic resistance. For example, an Egyptian review found *S. aureus* mortality risk is increased and MDR strains prevalent; limited surveillance hampers precise estimates. Overall, studies suggest that MRSA (often ST88 “African clone”) can account for 25–50% of *S. aureus* in some African hospitals (Lozano et al., 2016).

Due to the limited national surveillance of *S. aureus* among paediatrics few evidence are known on the prevalence of the *S. aureus* infections, however some retrospective studies conducted using hospital patients indicate that *S. aureus* is a leading pathogen in children. A retrospective study conducted by Oluwo et al. (2024) In Lagos, found that *S. aureus* was the most frequent bacterial isolate from children (32.1% of isolates) and Pseudomonas spp. was the least frequent pathogen isolated 7.1% (11/156) in all samples. Another study also showed that among paediatric cases of *S. aureus* infection, multidrug resistance was high. Similarly, a multicentre Nigerian study (2020–2022) of neonates with sepsis found *S. aureus* in 56% of culture-proven sepsis cases, with over half of those *S. aureus* isolates being MRSA; these studies highlight that *S. aureus*, including MRSA, causes substantial paediatric morbidity in Nigeria. (Mustapha et al., 2024).

Several other studies report a high prevalence of *S. aureus* among children in Nigeria. According to Iregbu et al. (2006), *S. aureus* accounted for 25–40% of isolates in paediatric infections at a tertiary hospital in Abuja. More recent data from Okon *et al.* (2020) indicate that *S. aureus* remains one of the leading bacterial pathogens isolated from children presenting with skin and soft tissue infections (SSTIs), bloodstream infections, and pneumonia.

Another study conducted by Onwubiko and Sadiq (2011) in Zaria found *S. aureus* in 36% of nasal swabs from school-aged children, indicating a high rate of asymptomatic colonization, which serves as a reservoir for infection and transmission. Similarly, Olayemi *et al*. (2021) reported a colonization rate of 30% among children in Ibadan, with higher prevalence among those with recent antibiotic use or frequent hospital visits.

The emergence of methicillin-resistant Staphylococcus aureus (MRSA) poses a significant threat to child health in Nigeria. A study by Ghebremedhin *et al.* (2009) found that 22% of S. aureus isolates from children in a Nigerian teaching hospital were MRSA, with resistance to multiple

antibiotics. The prevalence of MRSA among paediatric patients varies regionally but tends to be higher in urban hospitals compared to rural settings due to greater antibiotic exposure. Furthermore, Adewumi *et al.* (2022) identified community-associated MRSA (CA-MRSA) strains in over 15% of healthy school children sampled in Lagos, raising concern about the widespread dissemination of resistant strains outside hospital environments.

Following the prevalence of *S. aureus,* antimicrobial resistance of *S. aureus* strains isolated from Nigerian children is increasingly becoming resistant to therapy. Studies show high resistance rates to commonly used antibiotics such as ampicillin, tetracycline, and cotrimoxazole. For example, Kehinde *et al.* (2014) reported resistance rates exceeding 70% for penicillin among paediatric isolates in southwestern Nigeria. However, vancomycin and linezolid remain effective against most strains, although there are sporadic reports of reduced susceptibility. The widespread use of over-the-counter antibiotics and lack of antimicrobial stewardship programs exacerbate the resistance challenge (Okeke *et al.,* 2025).

The epidemiology of *S. aureus* among paediatrics population in Benin City (Edo State, Nigeria), recent cross-sectional survey in Benin City found extremely high rates of nasal *S. aureus* colonization and resistance in hospital attendees of all ages. Among 165 participants swabbed, *S. aureus* was recovered in 55.1%; remarkably, 91.2% of those isolates were MRSA (Elimian et al., 2023) Although this sample was mixed-age, it suggests heavy MRSA burdens in that urban Nigerian population. This reinforces concerns that local clonal MRSA strains are widespread.

Another Studies conducted by Ogefere et al. (2019) using samples in Benin City have demonstrated a high prevalence of nasal colonization by *S. aureus*, including methicillin-resistant strains (MRSA). Findings from the study showed that 42.3% of healthy students carried *S. aureus* in their nasal cavities, and among them, 37.2% were carriers of MRSA. Although this

study was conducted among tertiary students, it reflects widespread asymptomatic colonization among youth, which is likely to be similar in children due to shared environmental exposures and hygiene challenges.

Clinical isolates from paediatric and general hospital settings in Benin City reveal a concerning pattern. Ibadin et al. (2017) found that 44% of *S. aureus* clinical isolates were methicillin-resistant by phenotypic methods, and 38% harbored the mecA gene. Wound infections and bloodstream infections were prominent among children, especially those admitted to the University of Benin Teaching Hospital (UBTH). Obasuyi et al. (2013) previously reported the presence of MRSA in 11% of *S. aureus* isolates from hospital patients, with the use of molecular typing methods confirming the presence of multiple resistant clones.

Resistance to commonly used antibiotics is alarmingly high in Benin City. Ibadin et al. (2017) and Ogefere et al. (2019) reported high levels of resistance to penicillin, erythromycin, and cotrimoxazole. Gentamicin and ciprofloxacin retained moderate effectiveness, but vancomycin-resistant strains have also been detected. Oroboghae and Igweh (2021) found that approximately 4.5% of isolates were resistant to vancomycin, which is particularly concerning given its status as a last-resort antibiotic.

2.7 Risk Factors for *Staphylococcus aureus* Colonization and Infection

Several factors elevate the risk of *S. aureus* colonization and infection in children. Close contact with individuals colonized with MRSA, the presence of scrapes, cuts, or other skin injuries, tattoos, piercings, and a history of previous MRSA infection are significant risk factors for active MRSA infection (Stanford Medicine, 2025). MRSA infections are more prevalent in groups of people who spend considerable time in close proximity, such as children participating in sports teams, where transmission can readily occur via shared equipment, clothing, and direct skin-to-

skin contact (Stanford Medicine, 2025). Children attending childcare centers also face a higher risk of MRSA infection and can serve as a potential reservoir for the bacteria. Furthermore, CA-MRSA infections are more likely to occur in children under three years of age (Yang et al., 2023).

For MRSA colonization specifically, several factors are significantly associated with higher odds: female sex (Odds Ratio = 4.17), recent surgery (OR = 3.79), recent hospitalization (OR = 2.63), and prior antibiotic use (OR = 2.42). From the studies conducted by Yang et al. (2023), the correlation between antibiotic use and higher odds of MRSA colonization creates a concerning cycle in paediatric care. The very tools used to treat bacterial infections are simultaneously driving the selection and spread of resistant strains, including MRSA colonization. In paediatrics, where viral infections are common but frequently treated with unnecessary antibiotics due to parental pressure or clinician caution, this cycle is particularly troubling. This situation implies that reducing unnecessary antibiotic prescriptions is not merely about treating the current infection but also about preventing future colonization with resistant organisms, thereby mitigating the risk of subsequent resistant infections.

Immunocompromised children, such as those who are HIV-positive, are more frequently affected by *S. aureus* infections and are more susceptible to multidrug-resistant (MDR) phenotypes (Sharma and Gupta, 2022).

The age-dependent incidence of *S. aureus* infections, characterized by high rates in the first year of life, a low incidence through young adulthood, and a gradual rise with advancing age 1, suggests evolving host immunity and exposure patterns throughout childhood. CA-MRSA infections, for instance, are more likely to occur in children under three years old (AL-lade, 2023). This pattern indicates the particular vulnerability of infants, likely attributable to their immature immune systems and specific exposure risks, such as healthcare contact or close family

contact. The subsequent lower incidence in older children and young adults, before a later increase, suggests that developing immunity may offer some protection, or that exposure patterns shift. The later rise in incidence could be linked to increased healthcare exposure, underlying comorbidities, or lifestyle factors in older adults (Tony et al., 2015). This age-specific susceptibility implies that prevention and surveillance efforts should be particularly intensified during infancy and early childhood, recognizing these unique windows of vulnerability.

Environmental and foodborne routes also play a significant role in the epidemiology of *S. aureus* in children. Olayemi et al. (2021); Orofure and Igbinosa (2021) documented *S. aureus* contamination in frozen meat and suya (a local grilled meat snack), with MRSA strains exhibiting resistance to multiple antibiotics. These findings raise concerns about indirect exposure and infection risks in children who consume such foods or are in close contact with food vendors.

2.8 *Staphylococcus aureus* and Zoonotic Transmission

S. aureus circulates widely in animals. Many mammals, birds, and livestock carry *S. aureus*, and some strains are transmissible to humans. Livestock-associated MRSA (LA-MRSA, e.g. CC398) is established in many regions. In Nigeria, a systematic review found MRSA prevalence in farm animals of 4–38% (varying by species) (Peton and LeLoir, 2014). Notably, up to 53.9% of pig samples and 37.5% of poultry samples were MRSA-positive in some studies. Persons with frequent animal contact (farmers, butchers, veterinarians) had very high MRSA carriage (3.1–71.4%) (Gaddafi et al., 2024). These data indicate zoonotic reservoirs and risk: *S. aureus* strains (both MSSA and MRSA) circulate between humans and animals. Pets and livestock can harbor human-pathogenic clones. Thus, zoonotic transmission is a recognized factor in epidemiology (Lozano et al., 2018).

In Nigeria, several studies have highlighted S. aureus contamination in food animals and their products. For instance, Olayemi et al. (2021) reported MRSA strains in frozen meats sold in Benin City, indicating possible transmission from animals to humans through improperly handled food. Similarly, Olatoye et al. (2016) found *S. aureus*, including antibiotic-resistant strains, in raw milk and beef samples collected from retail markets. These findings suggest that poor hygiene practices, lack of veterinary oversight, and unregulated antibiotic use in animal farming contribute to the emergence and spread of zoonotic *S. aureus* strains in Nigeria.

2.9 Antimicrobial Susceptibility Testing

Given the ever-evolving resistance of *S. aureus*, empirical antibiotic prescribing is increasingly unreliable. Clinical microbiology advanced to address this uncertainty. In 1966, Sherris, along with Kirby, Bauer, and others, standardized the disk diffusion susceptibility test (Kirby–Bauer method), enabling reproducible phenotypic detection of antibiotic susceptibility (Sherris et al., 1966; NIH, 2024).

Without susceptibility testing, clinicians risk prescribing ineffective antibiotics, promoting further resistance and jeopardizing patient outcomes. For example, mecA-positive strains may show low-level phenotypic resistance detectable only through cefoxitin disk tests or PCR (NIH, 2024), and mecC-bearing strains may evade detection unless specific phenotypic assays are performed (Khoshnood et al., 2017).

Moreover, stewardship programs rely on AST (antimicrobial susceptibility testing) to optimize therapy, avoid unnecessary use of last-resort agents (like linezolid or vancomycin), and mitigate the spread of resistant clones. In the Spanish linezolid-resistant outbreak, stewardship reduced antibiotic pressure and halted further cases (Wired, 2010). Rapid molecular assays, such as PCR

detection of mecA, complement phenotypic methods and allow detection of resistance genes even before clinical resistance emerges, guiding early intervention.

The rise of MRSA has critical treatment implications in paediatrics. MSSA infections respond well to anti-staphylococcal beta-lactams (e.g. nafcillin, first-generation cephalosporins), which are generally more efficacious than vancomycin for MSSA. In contrast, MRSA cannot be treated with beta-lactams, necessitating agents like vancomycin (or teicoplanin) and linezolid, which have dependable activity against MRSA. Empirically, many guidelines recommend vancomycin for suspected invasive MRSA in children, while clindamycin (if local resistance is low) or trimethoprim-sulfamethoxazole (TMP-SMX) may cover community MRSA skin infections. In the Saudi paediatric study, all MRSA isolates were susceptible to vancomycin and linezolid underscoring these drugs as reliable options. However, clindamycin and TMP-SMX can fail if resistance is present. For example, the African meta-analysis found MRSA resistance rates of ~23.6% to clindamycin and 38.9% to TMP-SMX (Azzam et al., 2025). In Nigeria's Lagos children's hospital, *S. aureus* showed high resistance to commonly used antibiotics: ampicillin, amoxicillin-clavulanate, and cephalosporins (Oluwo et al., 2024). Thus, empirical therapy must consider local antibiograms.

Study conducted by Pinheiro et al., (2023) aimed at evaluating the changes in antimicrobial susceptibility testing of bacteria infecting children and mother in a neonatal intensive care unit. A total of 196 patients' medical records were analyzed. From this records; a total of 256 microorganisms were identified during this period. Antimicrobial susceptibility tests were performed on 196 (76.6%) clinical isolates. The exact binomial test showed that the distribution of Gram-negative bacteria was predominant. The most common microorganism was *Escherichia coli* (23%; n = 45), followed by *Staphylococcus aureus* (17.9%, n = 35), *Klebsiella pneumoniae*

(12.8%, n = 25*), Enterococcus faecalis* (7.7%, n = 15), *Staphylococcus epidermidis* (6.6%, n = 13) and *Pseudomonas aeruginosa* (5.6%, n = 11). *Staphylococcus aureus* was the predominant species among resistant bacteria. Among the antimicrobial agents tested, the following were resistant, presented on a descending scale: penicillin (72.7%, p = 0.001, Binomial test), oxacillin (68.3%, p = 0.006, Binomial test), ampicillin (64.3%, p = 0.003, Binomial test), and ampicillin/ sulbactam (54.9%, p = 0.57, Binomial test). Infections with S. aureus were 3.1 times greater in paediatrics and maternal units than in other hospital wards. The study concluded that despite the global reduction in the incidence of MRSA, an increase in MDR *S. aureus* was observed in their study.

Mzee et al. (2021) conducted a study on the prevalence of antimicrobial susceptibility and genotypic characteristics of *Staphylococcus aureus* in a Tanzania population. Findings from the systematic review showed that MRSA averaged a 21 % prevalence across all reviewed studies. Seven studies further characterized MRSA isolates, reporting elevated resistance (50–100 %) to clindamycin, erythromycin, and co-trimoxazole, in addition to the intrinsic β-lactam resistance.

Regarding the susceptibility of *S. aureus to* vancomycin, most studies reported 100 % susceptibility of *S. aureus* to vancomycin. However, three investigations (Geofrey et al. 2015; Kayange et al. 2010; Seni et al. 2019) observed vancomycin resistance exceeding 10%.

Phenotypic analysis and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* in Kenya conducted by Iliya et al. (2020), 138 clinical samples were collected from patients assessing care in the sampled hospital using standard bacteriological techniques. Methicillin resistance of Staphylococcus aureus was determined using the cefoxitin disk diffusion test. Out of 138 samples, 54 (39.1%) were found to have *Staphylococcus aureus* of which 22 (40.7%) were shown to be MRSA using the cefoxitin- based susceptibility test. Antibiotic susceptibility

testing using Kirby-Bauer technique was performed on all 54 isolates. The highest sensitivity was found in chloramphenicol 46 (85.2%) and lowest in penicillin-G 8 (14.8%). Multi-Drug Resistance (MDR) was reported in 35 (64.8%) of the 54 isolates of Staphylococcus aureus. All 22 MRSA strains were found to be MDR.

Similarly, study conducted by Oguda et al. (2022) on the prevalence and antibiotic susceptibility patterns of *S. aureus* isolated from wounds in diabetic patients. The isolates were identified through biochemical tests, and antimicrobial susceptibility was determined using the agar disk diffusion method. Of the 156 samples, 31 (19.87%) were positive for *S. aureus*, while 125 (80.13%) were negative. Among the positive isolates, 26 (10.48%) exhibited intermediate sensitivity, and 72 (29.03%) showed resistance to at least one antibiotic. More than half of the isolates were susceptible to the tested antibiotics. The highest susceptibility was observed for Cefoxitin (96.77%) and Clindamycin (80.65%), while Ampicillin demonstrated the lowest susceptibility (25.81%). The study established, 19.87% prevalence of S. aureus in wounds of diabetic patients at the outpatient diabetic clinic of MTRH, with most isolates showing susceptibility to Cefoxitin, Erythromycin, and Clindamycin. Regular surveillance, early screening, and re-evaluation of treatment options, particularly Ampicillin, are essential for effective management diabetic wound infections and to combat antibiotic resistance

Overall, high MRSA prevalence among paediatric patients (e.g. ~45% in one Saudi center Hadiah et al. (2024) and >50% of staphylococcal neonatal sepsis in Nigeria Oluwo et al. (2024) means that empiric therapy often must assume resistance. This has led to heavy reliance on vancomycin, which in turn raises concerns about nephrotoxicity and promoting “MIC creep.” The low rates of vancomycin/linezolid resistance seen (5–6%) are reassuring, but vigilance is needed. Antimicrobial stewardship is crucial: children on empiric MRSA therapy should be de-

escalated if cultures show MSSA or if clinical improvement occurs (Azzam et al., 2025). Additionally, failure to account for high MRSA rates can lead to treatment delays and worse outcomes. Conversely, overtreating presumed MRSA can overtly expose children to toxic drugs when unnecessary.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study setting

The study was conducted in Benin City, Edo State. Benin City is the capital city of the State and the most metropolitan city in Edo State. It is located in the southern part of the country, specifically within the Niger Delta region. The city encompasses several local government areas, including Oredo, Egor, and Ikpoba Okha, and is known for its rapid urbanisation and associated environmental and land management challenges. The state has a tropical or savanna climate with a typical hot and humid weather, characterised by temperatures ranging from 25ºC-30ºC (77 to 86 °F) (Weather Forecast, 2024). The inhabitants engage in various occupations, including civil jobs, agriculture, and other commercial activities. The city is served by several public and private healthcare facilities, including the University of Benin Teaching Hospital (UBTH), Central Hospital Benin, Stella Obasanjo Hospital, military and police hospitals, and numerous primary healthcare centres. UBTH, in particular, is a tertiary referral centre for the entire South-South region and parts of neighbouring states, providing specialised paediatric services and hosting a fully equipped microbiology laboratory capable of performing advanced antimicrobial susceptibility testing.

3.1 Study design

The study adopted a cross-sectional epidemiological design, which is a research design that can be used to determine the prevalence of a disease or condition within a population and to explore associations between exposures and outcomes.

3.2 Study the hospital and laboratory

The study was conducted at the University of Benin Teaching Hospital (UBTH), located in Benin City, Edo State, Nigeria. UBTH is a federal government-owned, multi-specialty tertiary healthcare facility situated in the Southern Senatorial District of Edo State. It has a bed capacity of approximately 910 beds, the hospital serves as a major referral centre for Edo State and neighbouring states in the South-South and South-West regions of Nigeria. UBTH comprises several clinical departments, including medicine, surgery, obstetrics and gynaecology, paediatrics, community health, and public health, among others. The Department of Paediatrics admits an average of 120 patients per month, managing a wide range of childhood illnesses, including infectious diseases. The department is actively involved in both undergraduate and postgraduate medical training.

3.3 Study participants

Participants include paediatric patients aged 2–17 years within UBTH with clinically diagnosed bacterial infections or paediatric patients who were suspected to eventually receive antibiotic treatments. These participants were selected using a pragmatic approach, the approach aids the inclusivity of the study in identifying and enrolling participants who were more generalizable and relevant to routine clinical or community health environments.

Specifically, the inclusion criteria include:

- Patients between the ages of 2-17 years
- Patients must be residents of Benin City

Exclusion criteria.

- Patients between (2-9 years) whose primary caregivers refused to grant consent were excluded from the study; patients (10-17 years) who refused to grant consent were excluded.

3.4 Sample size and sampling

The sample size for the proposed study was estimated based on the formula proposed by Kirkwood and Sterne (2010).

$$n = \frac{3.84\pi(1-\pi)}{w^2}$$

Where: n=required minimum sample size

π=proportion of AMR in children

w=precision of estimate (i.e., confidence interval).

Various study has ascertained the varying prevalence of AMR in paediatric populations in Nigeria, including the research conducted by Ughasoro et al. (2021); they noted a prevalence rate of 50% in their study. Similarly, the sample size for this present assumes a prevalence of 50% (π=0.5) and estimated 5% (w=0.05) with 95% certainty.

$$n = \frac{3.84x0.5x(1-0.5)}{0.05^2}$$

N=384 respondents

However, after adjusting for a 15% non-response

$$= \frac{100}{100 - 15} \times 384$$

$$= 451.77$$

Thus, the final sample size for the study would be 452 (~455) paediatric patients, however of this population a total of 65 samples will be collected.

3.5 Cross-sectional study

3.5.1 Priority pathogens

The WHO Bacterial Priority Pathogens List (BPPL) is a key tool for prioritising research and development investments and informing global public health policies to combat AMR (WHO, 2024). The 2024 list includes 15 pathogen families, categorised into Critical, High, and Medium priority tiers. Categorised based on the severity of their resistance and impact on global health, the critical priority resistant pathogens for children include antibiotic-resistant Gram-negative bacteria, such as *Enterobacteriaceae* spp. (carbapenem-resistant, extended spectrum beta-lactamase ESBL, or third-generation cephalosporin-resistant), *K. pneumoniae, Acinetobacter* spp., *Mycobacterium tuberculosis*, and *E. coli*). High-priority resistant pathogens include *Salmonella enterica* serotype Typhi (fluoroquinolone-resistant) and *Shigella* spp. (fluoroquinolone-resistant), *Enterococcus faecium* (vancomycin-resistant), *P. aeruginosa* (carbapenem-resistant), Non-typhoidal *Salmonella* (fluoroquinolone-resistant), and *S. aureus* (methicillin-resistant) (WHO, 2024; Sati et al., 2025). Furthermore, medium-priority resistant pathogens, such as Group A (macrolide-resistant) and Group B (penicillin-resistant) *Streptococci*, *Streptococcus pneumoniae* (macrolide-resistant), and *Haemophilus influenzae* (ampicillin-resistant), will be assessed, given that they disproportionately affect infants and young children, especially in resource-limited settings (Sati et al., 2025). While antibiotic resistance is a growing concern, particularly with methicillin-resistant *Staphylococcus aureus* (MRSA), understanding

the epidemiology and risk factors is crucial for effective prevention and management strategies. Therefore, this study will prioritise resistance in *S. aureus.*

3.5.2 Training

The study involved various professionals, including microbiology scientists, data/sample collectors, and veterinarians, to collect data and samples from humans, related to the respective patients who gave consent to participate in the study. The training was conducted at the study site for pragmatic reasons, such as finance. The scope of the training covered overview of the study protocol, data entry via the Open Data Kit (ODK) Collect App on an Android tablet or smartphone, sample collection from various sources, transportation to the laboratory, and data integrity checks.

3.6 Data and sample collection

Based on the predefined study inclusion criteria, study data informed potential participants' caregivers about the study, including the ethics requirements, using both verbal and written approaches. Privacy was maintained during the information collection process, and no treatment was delayed or denied due to enrolment in the study. Caregivers were offered sufficient time to consider their participation in the study before providing informed written consent.

Trained data collectors in the study hospital administered the questionnaire to caregivers, reviewed hospital records for clinical history, and monitor antibiotic prescriptions. They were also being responsible for conducting physical examinations of patients and collecting clinical samples (rectal swabs and nasal swabs). While rectal and nasal swabs were collected from all patients for the assessment of antibiotic carriage, presenting with suspected UTI and sepsis.

3.7 Urine and rectal swab samples

Urine samples were obtained and cultured on the same day in children suspected of having a UTI, while the culture of rectal swabs were performed regardless of the diarrhoea status. However, diarrhoea episodes in patients were recorded in terms of type (severe, persistent, bloody diarrhoea) and duration. In toilet-trained children, urine was obtained by clean voided mid-stream urine sampling. For those not toilet-trained, samples were obtained by clean-catch voided urine or catheterised urine, where appropriate. The sample was collected into a sterile universal container, labelled, and sent immediately to the laboratory for prompt microbiological assessment and processing. In brief, the gross and microscopic appearances were examined first. Then, an estimated 10 μL of urine was inoculated onto Cystine Lactose Electrolyte Deficient and Blood agar (BA) plates containing 5-10% blood. The plates were then incubated in ambient air at 37°C for 24–48 hours. In the case of bacterial growth, the colonies were to be identified using standard bacteriological methods. A count of at least 10^5 colony-forming units/ml (CFU/ml) was considered diagnostic of UTI.

Collected rectal swabs were cultured on appropriate media for the detection of enteric pathogens, following standard laboratory procedures. The rectal swabs were inoculated on MacConkey agar supplemented with 5mg/L of cefotaxime and incubated at 37ºC for 24 hours. Emergent Gram-negative rods that are lactose-fermenting colonies were identified using API20E kits according to the manufacturer's instructions. The organisms were then stored in Luria broth with 20% glycerol at 80 °C for further analysis.

3.8 Blood Samples Collection in Paediatric Patients

The collection of blood for culture in paediatric patients was performed using strict aseptic techniques to ensure accurate detection of bloodstream pathogens while minimizing contamination. The recommended blood volumes varied according to the child's age and weight.

For neonates, 0.5–1 mL of blood was collected per culture set, while infants and small children provided 1–3 mL. Older children and adolescents contributed 4–10 mL, following the fill lines indicated on paediatric aerobic culture bottles. To safeguard against iatrogenic anaemia, institutional safety limits were observed, with a maximum of 3 mL/kg/day across all laboratory draws and 10 mL/kg/week. Where clinically feasible, one to two culture sets were collected from separate venipuncture sites to enhance diagnostic yield.

Before specimen collection, patient identity was verified using two identifiers, and the procedure was explained to the parent or guardian, with assent obtained from the child where appropriate. Hand hygiene was strictly performed, and sterile gloves were donned before beginning the procedure. A suitable vein was identified with the application of a tourniquet. The venipuncture site was first cleaned with an alcohol wipe, followed by skin antisepsis using 2% chlorhexidine in 70% isopropyl alcohol for children above two months, or povidone-iodine for neonates and younger infants. Transport and handling of blood culture bottles were carried out under recommended conditions. Samples were maintained at room temperature and delivered to the microbiology laboratory immediately, preferably within one hour of collection. Refrigeration of blood culture bottles was strictly avoided, as it can compromise microbial viability and growth.

3.9 Nasal Swab Collection in Paediatric Patients

Nasal swabs were collected to determine *Staphylococcus aureus* colonization among paediatric participants. The anterior nares were chosen as the sampling site, as this location is the principal reservoir for *S. aureus* carriage in children. A sterile cotton wool swab with transport medium was used for each participant.

The swab was gently inserted into the first anterior naris to a depth of approximately 1–2 cm, taking care not to cause discomfort or trauma. It was rotated along the mucosal surface for 5–10

seconds, completing at least five circular motions to maximise recovery of nasal flora. Using the same swab, the procedure was repeated in the contralateral naris to ensure adequate sampling.

After collection, the swab was transported to the laboratory in Stuart's medium at ambient temperature. Within 2 hours of collection, swabs will be inoculated directly onto Mannitol Salt Agar (Oxoid Ltd., Basingstoke, UK) and blood agar. Each specimen was labelled with the participant's identifier, the type of specimen, and the date and time of collection.

3.10 Bacterial strain identification

Bacterial colonies from all clinical were identified using conventional laboratory methods, including colonial morphology and Gram stain, and confirmed by an appropriate biochemical test. To identify members of *Enterobacteriaceae*, the API 20 E system was used according to the manufacturer's instructions. A catalase test was performed on Gram-positive cocci to differentiate between *Staphylococcus* and *Streptococcus* species. The test was conducted as described elsewhere (CISI, 2023). Further identification of the catalase-positive Gram-positive cocci, *Staphylococcus*, was done using API *Staph* according to the manufacturer's instructions. Isolates suspected to be *Haemophilus* spp. were identified using the X and V discs test. Similarly, Gram-positive diplococci bacteria identified during Gram staining were further identified using the optochin test on blood agar (Cheesbrough, 2006).

3.11 Antimicrobial susceptibility testing

Clinical isolates of *Staphylococcus aureus* were tested using the Kirby–Bauer disk diffusion method on Mueller–Hinton (MH) agar (Adhikari et al., 2017). A 0.5 McFarland standard suspension was prepared from fresh colonies and evenly spread on MH agar (ASM, 2009). Antibiotic disks were applied, and plates were incubated at 35 ± 2 °C for 16–18 hours. Inhibition

zones were measured and interpreted as Susceptible, Intermediate, or Resistant according to CLSI breakpoints (Qodrati et al., 2022).

Inducible clindamycin resistance was detected using the D-test, while isolates showing cefoxitin (30 µg) zone diameters ≤21 mm was classified as MRSA. Vancomycin susceptibility was determined by broth microdilution or E-test, as disk diffusion is unreliable for glycopeptides (Qodrati et al., 2022). All results were interpreted in accordance with CLSI standards (Adhikari et al., 2017).

3.11.1 Determination of MDR, extensively drug-resistant (XDR), and pandrug-resistant (PDR) among bacterial strains recovered

The criteria, as defined by Magiorakos *et* al. (2012), were adopted in this study. MDR bacteria would be non-susceptible to at least 1 agent in ≥3 antimicrobial categories, XDR bacteria were non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories, and PDR strains were non-susceptible to all agents in all antimicrobial categories.

3.11.2 MRSA screening

All identified *S. aureus* isolates recovered in this study were screened for methicillin resistance using a cefoxitin disc (30 µg), and interpreted as described by the Clinical Laboratory Standard Institute (CLSI, 2025). All MRSA were stored at -80ºC.

3.11.3 Extended-spectrum beta-lactamase (ESBL) screening

All Gram-negative bacilli recovered from MacConkey agar supplemented with 5 mg/L cefotaxime were screened for ESBL production using the double-disk synergy test (DDST) as described by Rahman et al. (2021). The following antibiotic discs were employed: amoxicillin-clavulanic acid (AMC) (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), and cefepime (30 µg), placed on Mueller–Hinton agar (MHA) plates. Briefly, colonies of the test organisms were emulsified in sterile distilled water, and the turbidity was adjusted to match a 0.5 McFarland

standard. The standardised inoculum will then be uniformly swabbed over the surface of MHA plates as per the standard disk diffusion protocol outlined in the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2023). The AMC disc will be positioned at the Centre of the plate, with the other discs placed 20 mm (centre-to-centre) from the AMC disc. Plates were incubated at 37 °C for 18–24 hours. ESBL production was also inferred for visible expansion of the zone of inhibition between any of the cephalosporin discs and the AMC disc, indicating synergy between the β-lactam and β-lactamase inhibitor (Rahman et al., 2021).

3.11.4 Carbapenemase test

All Gram-negative bacilli that were resistant to any of the carbapenems (imipenem or meropenem) in the disc diffusion test would be subjected to carbapenemase screening test using the modified Carbapenemase inactivation method (mCIM) as described in CLSI, 2025. Briefly, 1 µL loopful of Enterobacteriaceae or 10 µL loopfuls *of P. aeruginosa* from blood agar plates were emulsified in 2 mL trypticase soy broth (TSB). A meropenem disk would then be immersed in the suspension and incubated for a minimum of 4h at 37ºC. A 0.5 McFarland suspension of the indicator strain- *E. coli* ATCC 25922, prepared in saline using the direct colony suspension method, would thereafter be inoculated on an MHA plate using the routine disk diffusion procedure as in the ESBL screening test. The meropenem disk would then be removed from the TSB and placed on an MHA plate previously inoculated with the *E. coli* ATCC 25922 within 15 minutes of inoculation of the plate. Plates would then be incubated at 37ºC in ambient air for 18-24 h. An inhibition zone diameter of 6–15 mm or colonies within a 16–18 mm zone was considered to be a positive result. In contrast, a zone of inhibition of 19 mm would be considered to be a negative result (CLSI, 2025).

3.12 Duration of data collection

The data collection protocol spanned a period of 3 months (June- August 2025).

3.13 Outcome variables and covariates

The primary outcome variable was the proportion of patients, among the total study participants, carrying resistant bacterial strains, including *Staphylococcus aureus* resistant to methicillin (MRSA) and multidrug-resistant (MDR) *Escherichia coli*. Classification of resistance followed standard definitions: multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) (Magiorakos et al., 2012).

The secondary outcome variables include;

- Prescription patterns of antibiotics were prescribed to all study participants.
- Bacteriological profile of all participants, detailing the bacterial species isolated from various clinical specimens.
- Factors associated with carriage of resistant bacteria, including the proportion and odds ratios comparing patients with and without resistant isolates.
- Indirect costs associated with carriage of resistant bacteria, such as additional diagnostic tests, prolonged hospital stays, and lost productivity.

Covariates will include sociodemographic characteristics (age, sex, residence, socioeconomic status), clinical variables (underlying medical conditions, hospitalisation history, prior antibiotic use), and behavioural factors relevant to antimicrobial resistance risk.

3.14 Data management and analysis

The data collected were downloaded from the ODK cloud-based database. After data cleaning and validation, the final dataset was anonymised before analysis. The missing indicator approach was used to handle missing data. Isolates with intermediate or resistant antibiotic susceptibility results were classified as resistant strains (non-susceptible) during data analysis.

Descriptive statistics for the study participants and isolates for the overall population were presented by hospital, age, and sex, where appropriate. Categorical data were assessed using the chi-square or Fisher's exact test. Continuous data was assessed using the student's t-test or the Mann-Whitney U-test. Proportions were not calculated with fewer than ten isolates in a category. To identify factors associated with the carriage of resistant bacteria, univariable and multivariable logistic regression analyses were conducted; findings were presented in terms of adjusted odds ratios and 95% confidence intervals. A multicollinearity assessment was done using the inflation variance factor to ensure that variables are not collinear. A $p< 0.05$ was considered statistically significant, and all data analyses were conducted in STATA 16.

3.15 Limitations of the methodology

The proposed study has some limitations. First, due to limited resources, resistant bacterial isolates from patients, were not immediately genotyped. Such analysis would have provided clearer insights into antimicrobial resistance (AMR) transmission across human. However, the laboratory's secure storage system ensures that these isolates are safely preserved for future genotypic analysis when resources permit.

Second, the cross-sectional design restricts our ability to determine the clinical burden of AMR, as infection outcomes vary with study duration. For instance, mortality rates reported three months post-infection are often higher than those recorded only during hospitalization (van Duin and Paterson, 2016). Despite this, the planned qualitative component will enrich the quantitative data by offering deeper understanding of childhood AMR and its broader effects on patients, families, and communities.

3.16 Study monitoring and quality assurance

An internal data monitoring plan was developed across all participating sites to ensure adherence to ethics requirements, completeness and accuracy of data entry, and, if applicable, isolation of collection and storage. The core research team members conducted regular audits of the data entered and raised queries when necessary. Collated reports were generated and presented to the study hospital regularly to monitor progress and the quality of data collected.

3.17 Ethics approval

The study received ethical approval from the University of Benin Teaching Hospital Health Research Ethics Committee (reference number: ADM/E 22/A/VOL. VII/14831247). Participants were given informed consent after understanding the study's social and scientific values, benefits, risks, privacy, confidentiality, scientific validity, and post-enrolment participant protection, including withdrawal. All data were encrypted using national data protection and privacy regulations to avoid unauthorised access to the clinical samples and data. Authorised data records were kept in a locked file cabinet and accessible only to the core research team. However, other researchers may be granted access to the anonymised data for analysis on a reasonable request to the corresponding author. Clinical samples were destroyed immediately after analysis. However, bacterial isolates were stored for further characterisation, resources permitting.

CHAPTER FOUR

RESULTS

Table 4.1 presents the socio-demographic distribution of paediatric patients whose nasopharyngeal and rectal samples were collected for antimicrobial susceptibility testing within Benin City, Edo State. The patients included both in-patients (admitted to the University of Benin Teaching Hospital) and out-patients.

A total of 67 paediatric participants were assessed for this study, 63(94.0%) of the respondents were in-patients, while only 4 (6.0%) were out-patients. In terms of sex distribution, males constituted the largest proportion of the study population, with 45 in-patient males (67.2%), compared to 18 in-patient females (26.9%) and 4 out-patient females (6.0%).

The mean age varied across categories. Female out-patients had a mean age of 30.5 months, indicating a much younger subgroup. In contrast, female in-patients had a higher mean age of 117.5 months, while male in-patients had a mean age of 98.6 months.

Table 4.1: Socio-Demographic Frequency of the Paediatrics Patient

What is the patient's sex?	What is the patient category?	Count	Mean_Age (Months)
Female	Out-Patient	4	30.5
Female	In-Patient	18	117.5
Male	In-Patient	45	98.6

The data presented in Figure 4.1 show the distribution of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CoNS) isolates recovered from rectal and nasopharyngeal samples. A total of 53 *Staphylococcus* isolates were identified, comprising 36(67.9%) *S. aureus* and 17(32.1%) Coagulase negative *Staphylococcus aureus* (CoNS). Majority of the isolates were obtained from nasopharyngeal samples, which yielded 35 *S. aureus* and 17 CoNS isolates. Only a single *S. aureus* isolate was recovered from a rectal sample, while no CoNS were detected from rectal specimens.

The antimicrobial resistance pattern of *Staphylococcus aureus* isolates, as shown in Figure 4.2, reveals a high prevalence of multidrug resistance (MDR), accounting for 53.10% of the isolates. This indicates that more than half of the *S. aureus* isolates demonstrated resistance to three or more classes of antibiotics. While, 15.60% of the isolates exhibited low-level resistance, implying that these strains were resistant to only one or two antimicrobial agents. The other 31.20% of the isolates were susceptible.

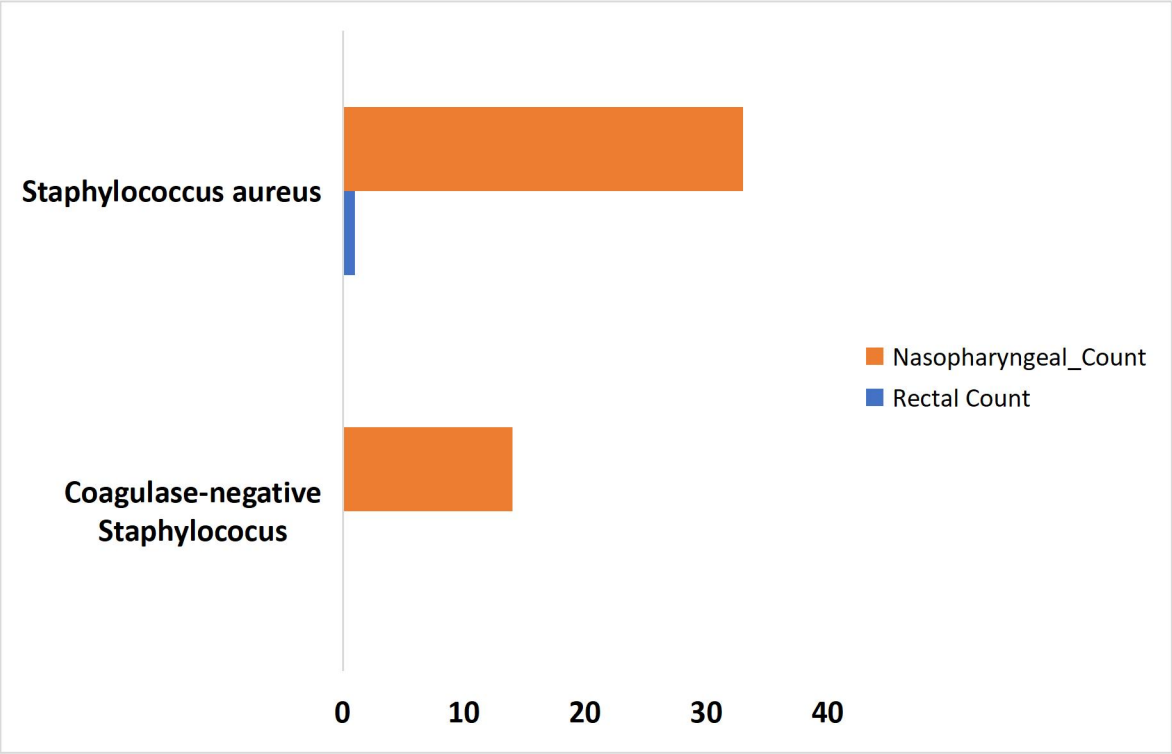

Figure 4.1: Distribution of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CoNS)

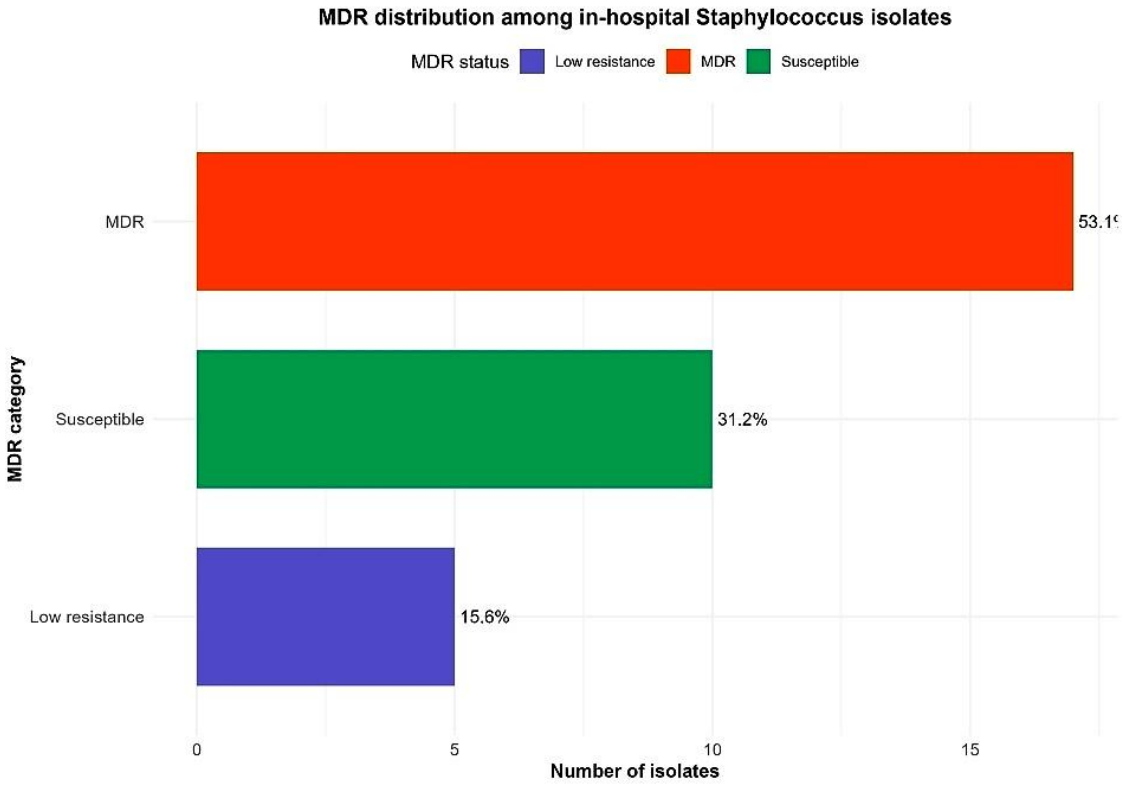

Figure 4.2: Multi Drug Resistance (MDR) among in-hospital *Staphylococcus* isolates

Figure 4.3 shows the antimicrobial susceptibility pattern of *S. aureus.* The antimicrobial susceptibility profile of *Staphylococcus aureus* isolates, indicated a high level of susceptibility to majority of the antibiotics; including amikacin, amoxicillin-clavulanic acid, tigecycline, meropenem, levofloxacin, erythromycin, cefuroxime, and tetracycline.

However, *S. aureus* exhibited resistance to some of the antimicrobials treatments. Notably, resistance was detected against sulfamethoxazole-trimethoprim, ciprofloxacin, cefoxitin, and clindamycin (Dalacin-C). Among these, resistance to cefoxitin is of particular clinical importance as it is commonly used as a surrogate marker for methicillin resistance, suggesting the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains within the tested population.

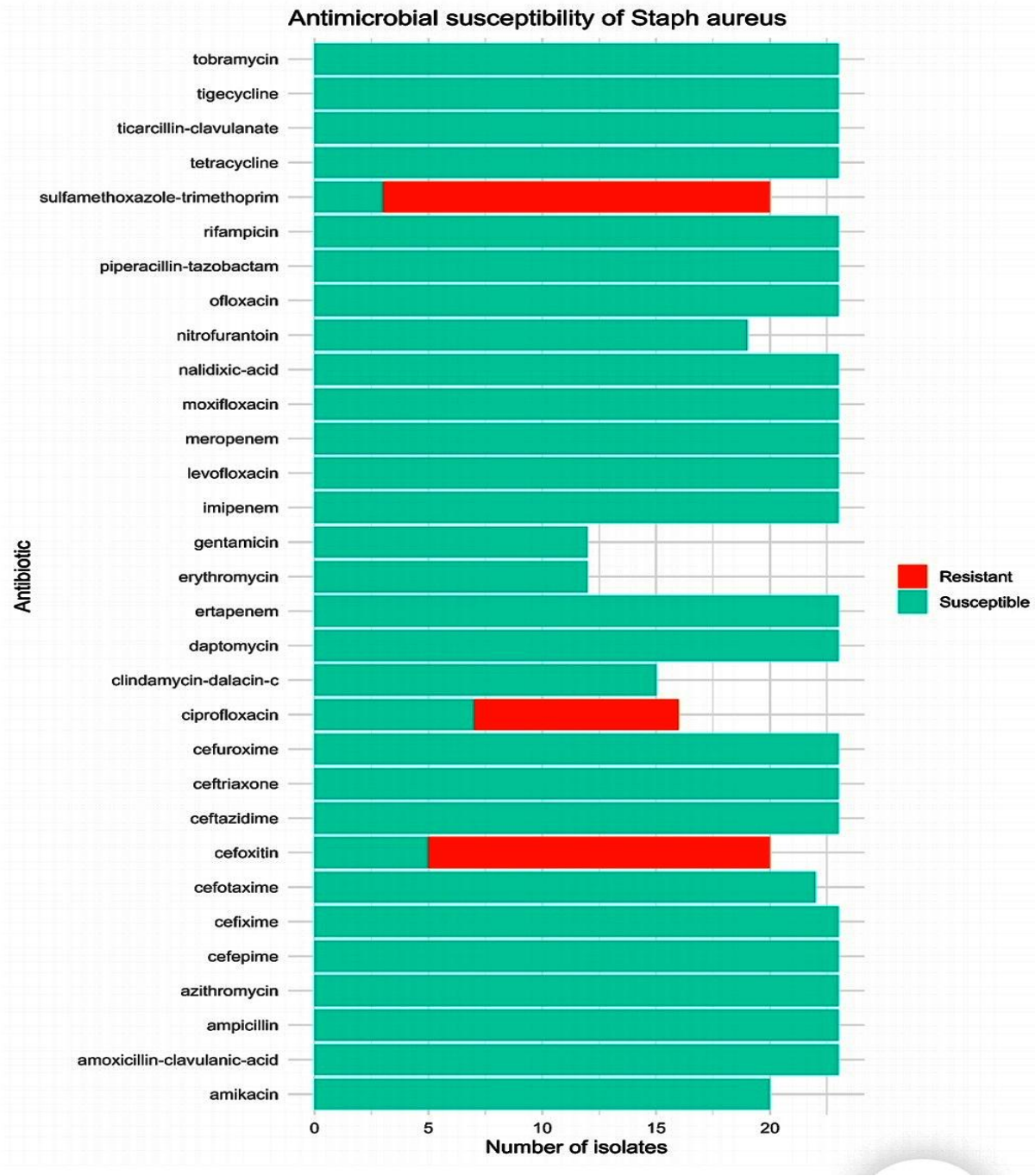

Figure 4.3: Antimicrobial Susceptibility Patterns in *Staphylococcus aureus* among hospitalises children

The antimicrobial resistance patterns in *Staphylococcus aureus* among hospitalized children is presented in Figure 4.4. Majority of the isolates exhibited a high level of susceptibility to most tested antibiotics, indicating that these agents remain largely effective for clinical management.

Resistance was most pronounced against cefoxitin, cefazidime, ciprofloxacin, erythromycin, and sulfamethoxazole-trimethoprim, where significant proportions of isolates were resistant (ranging from approximately 45% to 80%). The high resistance to cefoxitin is particularly concerning, as it serves as a phenotypic marker for methicillin-resistant Staphylococcus aureus (MRSA), indicating that MRSA strains are present among the paediatric patients studied. Moderate resistance was also detected against clindamycin and rifampicin, each showing partial resistance patterns.

In contrast, complete susceptibility (100%) was observed for several antibiotics including amikacin, tigecycline, piperacillin-tazobactam, ticarcillin-clavulanate, levofloxacin, meropenem, and imipenem, highlighting their potential as effective treatment options for S. aureus infections in this population. The absence of resistance to these agents may be attributed to their restricted use or lower selection pressure in paediatric care.

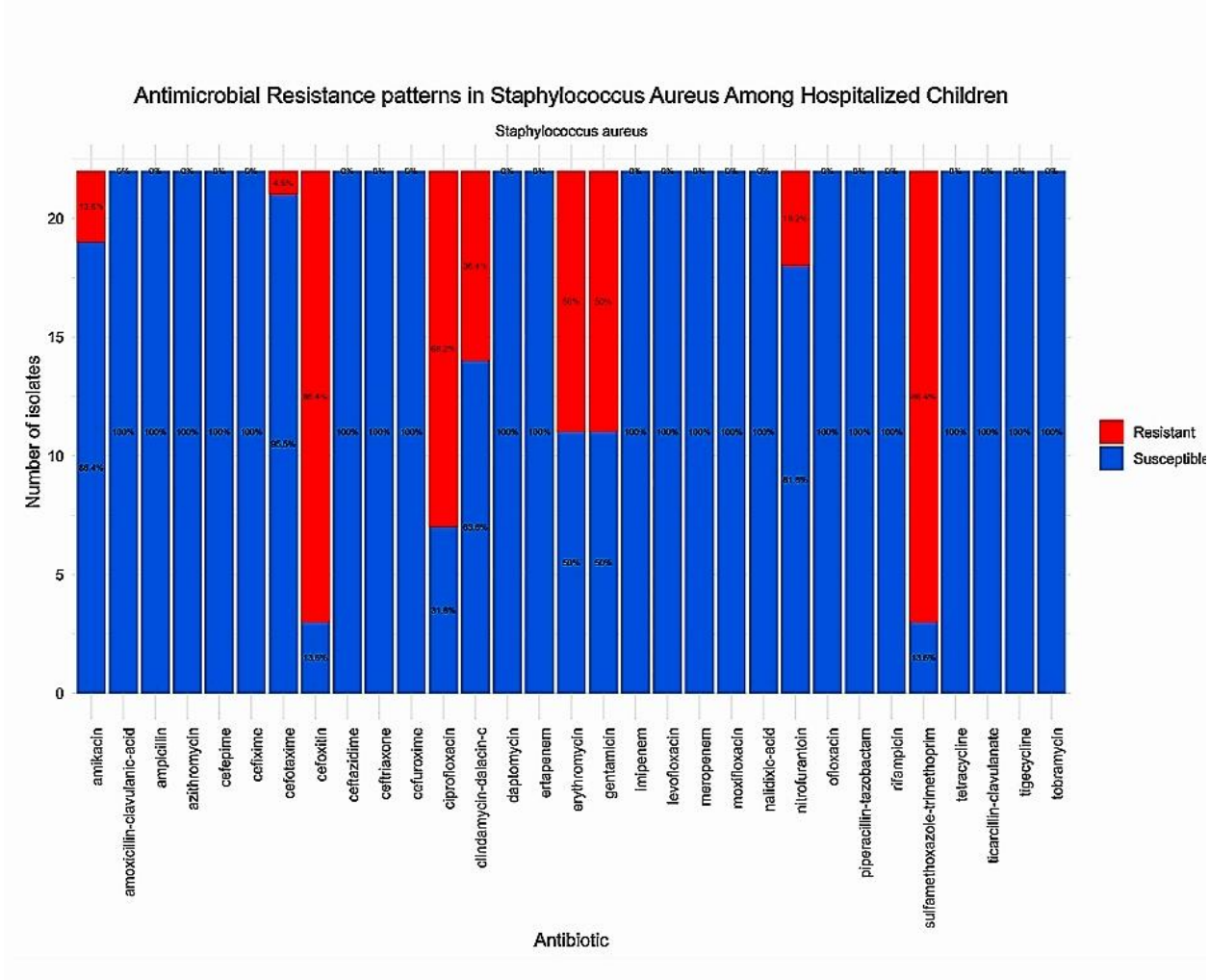

Figure 4.4: Antimicrobial Resistance Patterns in *Staphylococcus aureus* among hospitalises children

Figure 4.5 illustrates the distribution of antimicrobial resistance scores among Staphylococcus isolates obtained from hospitalized children across different age groups. The resistance score represents the number of antibiotics to which each isolate exhibited resistance.

The results indicate variation in resistance patterns across age groups. The highest resistance scores, ranging from 6 to 8 antibiotics were predominantly observed among the 1–4 years (toddler) and 5–9 years (child) age groups. In contrast, isolates from infants (<1 year) and older children (10–14 years and 15–17 years) demonstrated relatively lower resistance scores, with most isolates resisting fewer than 3 antibiotics.

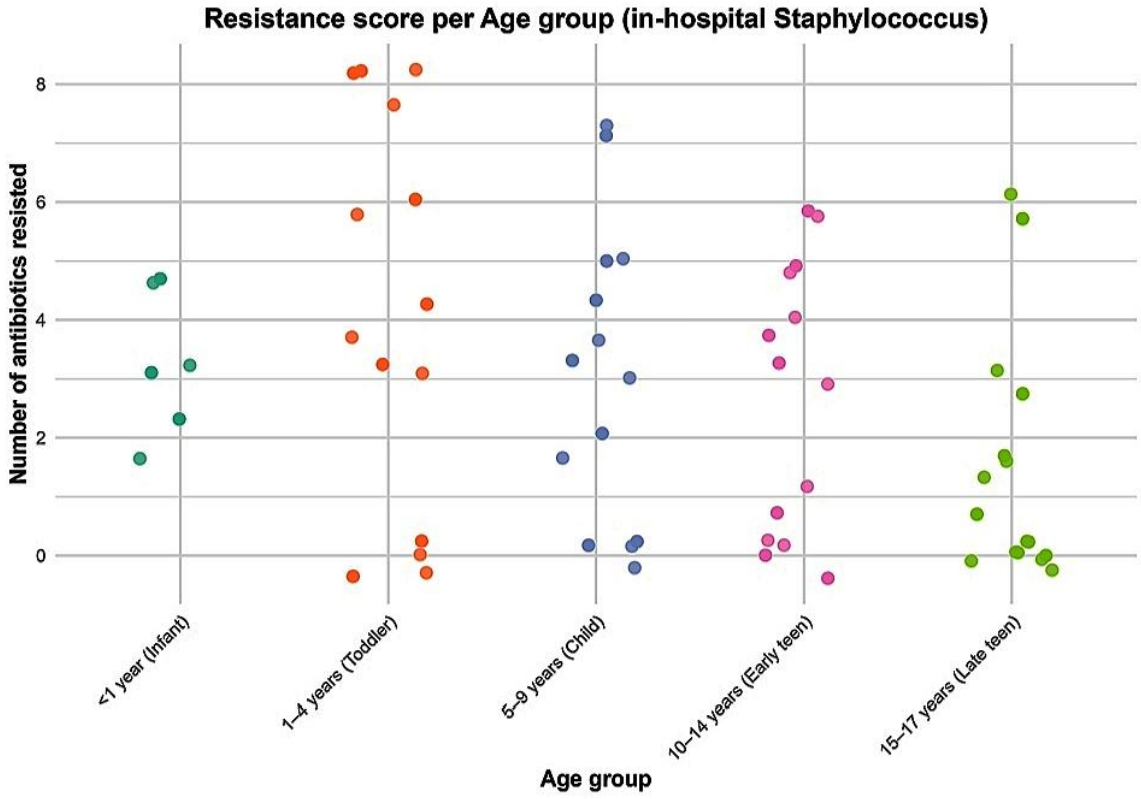

Figure 4.5: Antimicrobial Resistance Score per age group

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Staphylococcus aureus remains a leading cause of bacterial infections in both adult and paediatric populations (Sutter et al., 2016). The persistent rise in methicillin-resistant *S. aureus* (MRSA) prevalence over decades underscores the urgency of investigating antimicrobial susceptibility patterns, particularly in vulnerable paediatric cohorts the primary objective of this study.

The progressive emergence and rapid dissemination of antibiotic-resistant *S. aureus* and its association with the use and consumption of antibiotics, constitute a major health concern and have been considered a global crisis (Shagufta and Jayaraji, 2010). In this study a total of 53 staphylococcal isolates were identified, comprising 36(67.9%) *S. aureus* and 17(32.1%) coagulase-negative *staphylococci* (CoNS). This distribution aligns closely with findings reported by Shibabaw et al. (2014). Although CoNS are often dismissed as contaminants, they are increasingly recognized as significant pathogens in immunocompromised hosts, particularly in nosocomial bloodstream infections (Farzana et al., 2008). Moreover, methicillin-resistant CoNS (MRCoNS) are globally prevalent and may serve as reservoirs for resistance gene transfer to MRSA.

In this study the nasopharynx emerged as the predominant site of *S. aureus* colonization, consistent with its established role as a commensal of the upper respiratory tract and a reservoir for opportunistic infection. In contrast, rectal carriage was infrequently observed, suggesting limited gastrointestinal colonization in this paediatric cohort. These findings corroborate those of Shibabaw et al. (2014), who similarly reported high nasal carriage rates of MRSA among children. The predominance of MDR strains is a matter of clinical concern, as it limits

therapeutic options and complicates the management of *S. aureus* infections. This finding underscores the importance of antimicrobial stewardship programs, regular resistance surveillance, and the judicious use of antibiotics to curb the further emergence and spread of resistant *Staphylococcus* strains.

Overall, these findings indicate a mixed resistance profile, with over half of the *S. aureus* isolates showing multidrug resistance (MDR), consistent with the data summarized in Figure 4.4. The findings of isolate showing MDR is synonymous with the findings of Agbo *et al.* (2024), where ninety-eight (98) isolates of *S. aureus* obtained from both rectal, nasal and wound swabs showed resistance to different β-lactam antibiotics and fluoroquinolones including erythromycin, gentamicin, and oxacillin. Agbo et al. (2024) noted that the resistance could be attributable to the presence of the Erm gene family encoding the methylase, which is responsible for the methylation of adenine in the 23 S rRNA ribosomal subunit. Furthermore, in this study no strains resistance was observed to vancomycin compared to the findings of several other studies that reported such occurrences (McGuinness *et al.,* 2017). Similar, results were reported by Gurung *et al.* (2020), who reported that MRSA isolates showed 100 and 0% resistance to penicillin and vancomycin, respectively. High-level resistance to sulfamethoxazole-trimethoprim, ciprofloxacin, and clindamycin further complicates empiric therapy. These patterns underscore the critical need for ongoing resistance surveillance, antimicrobial stewardship, and infection prevention and control (IPC) measures to mitigate the spread of resistant strains in healthcare settings (Salam et al., 2023).

Age impacts antimicrobial resistance (AMR) in patients through a complex relationship with a patient's immune system, antibiotic use, and the specific type of bacteria. Older adults have a weaker immune system and tend to receive more antibiotics, increasing their risk for AMR

infections. However, some resistance patterns peak in younger or middle-aged adults depending on the specific bacteria and antibiotic, and the link is not uniformly higher in older patients for all pathogens (Knight, 2024). Age and sex were also found to be correlated with antimicrobial resistance pattern in an individual (Knight, 2024). The findings of this study on the resistance pattern of *Staph. aureus* revealed a peak resistance during early and middle childhood, followed by a gradual decline in older paediatric patients. Similar findings were reported in the research of Pillay *et al.* (2024), in their study too they observed similar trend such that a higher number of antimicrobials resistance per child (1.7-1.9 per patient) was higher in neonates and infants compared to children 6-12 years old (1.4 per patient). Further analysis also showed that the incidence risk ratio (IRR) of HAI was higher in neonates and infants (IRR 2.13; 95% CI 1.23-3.70, IRR 2.20; 95% CI 1.40-3.45, respectively) compared to 6- to 12-year-olds. These findings highlight the importance of age-specific surveillance and antibiotic stewardship programs to limit the development and spread of resistant Staphylococcus strains among hospitalized children.

5.2 Summary of the Findings

The study was conducted using clinical samples of both outpatient and inpatient, which the isolated *Staphylococcus aureus,* showed that the positive strains were higher among the paediatric population compared to the coagulase negative *S. aureus* isolates in this test. A total of 53 staphylococcal isolates were recovered from paediatric clinical samples (inpatient and outpatient), with *S. aureus* (67.9%) predominating over CoNS (32.1%).

The nasopharynx was the primary colonization site; rectal carriage was rare. Over 50% of *S. aureus* isolates exhibited multidrug resistance (MDR). High resistance was observed to sulfamethoxazole-trimethoprim, ciprofloxacin, cefoxitin (indicating MRSA), and clindamycin.

No resistance to vancomycin was detected. Resistance burden peaked in early and middle childhood, declining with age.

5.3 Conclusion

The study identified mild level of antibiotic resistance in the studied clinical *S. aureus* isolates. It raises the alarm of an impending antibiotic resistance crisis in the region. The study, however, recommends the rational drug use and adequate combination therapies, continuous bacterial resistance pattern surveillance, the establishment and deployment of efficient policies via constructive efforts of relevant stakeholders towards addressing the associated antibiotics resistance and their implications., and the advancement of research, including the development of rapid and reliable bacterial resistance screening techniques essential to instituting appropriate therapies/interventions.

5.4 Recommendations

Based on the findings, the following actions are strongly recommended:

1. Implement robust antimicrobial stewardship programs (ASP) in paediatric units, emphasizing de-escalation, duration limits, and prescriber education.
2. Establish routine, age-stratified AMR surveillance using standardized methods (e.g., CLSI/EUCAST) to track resistance trends and guide empiric therapy.
3. Strengthen infection prevention and control (IPC) practices, including:
4. Active screening of high-risk patients (e.g., neonates, ICU admissions)
5. Decolonization protocols for MRSA carriers
6. Hand hygiene compliance monitoring
7. Promote rational antibiotic use through diagnostic stewardship (e.g., avoiding treatment of colonization) and combination therapy only when evidence-based.

8. Invest in molecular diagnostics for rapid detection of resistance determinants (e.g., mecA, erm genes) to enable targeted therapy.
9. Foster multisectoral collaboration among clinicians, microbiologists, pharmacists, and policymakers to develop evidence-based guidelines and enforce regulatory oversight of antibiotic sales.

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