Detection of Methicillin-Resistant *Staphylococcus aureus* in Clinical Samples at Kenyatta National Hospital Using Phenotypic and Molecular Methods

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DECLARATION

I, Antony Kuria, do hereby affirm this as my original work that has not been presented to any other University for examination or award of a degree. I also declare the content therein as the product of my work and where other people's work has been used, this has adequately been acknowledged and referenced per the University of Nairobi's requirements.

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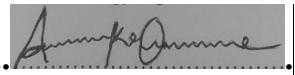
Signed…….  **.....Date.....13/12/ 2023.....**

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ABBREVIATIONS AND ACRONYMS

MRSA	Methicillin-resistant *Staphylococcus aureus*
CA-MRSA	Community-Acquired Methicillin resistant *Staphylococcus aureus*
CLSI	Clinical Laboratory Standards institute
DNA	Deoxyribonucleic acid
MSSA	Methicillin-Sensitive *S. aureus*
MSA	Mannitol salt agar
orfX	Open reading flame of Chromosome
PCR	Polymerase chain reaction
PVL	Panton-Valentine leucocidin
SCCmec	Staphylococcal cassette chromosome mec
IBM SPSS	Statistical Software application for academic and research studies
WHO	World Health Organization
WHONET	World Health Organization Network (for antimicrobial surveillance)
AM-A	Amplification mix A
AM-B	Amplification mix B
AMR	Antimicrobial resistance
UK	United Kingdom
MIC	Minimum inhibitory concentration

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global problem with a prevalence of between 29 and 35% reported worldwide. There is great variation in the epidemiology of MRSA at global and regional levels. Locally the reason for the variation in reported prevalence has been attributed to a lack of standardized methods for the detection of MRSA with some facilities using nonspecific methods such as coagulase to confirm *S. aureus*. This study aims to compare the diagnostic accuracy of the Vitek-2 system with the cefoxitin disc test in the detection of MRSA prevalence at Kenyatta National Hospital in Kenya using PCR as the gold standard.

Methods: A cross-sectional study design was used where all non-duplicate *Staphylococcus aureus* isolates from various specimen types from patients at Kenyatta National Hospital were collected. *S. aureus* isolate purity was ascertained by subculture before they were identified and tested for their sensitivity to various antibiotics according to Clinical Laboratory Standards Institute (CLSI) guidelines using the Vitek-2 System. MRSA isolates were confirmed using cefoxitin disc diffusion and GenoType MRSA PCR method for the detection of *the mec*A or *mec*C gene. Data on antibiotic sensitivity testing obtained from the Vitek-2 database was entered into WHONET software where it was analyzed and presented in tables and charts. Minimum inhibitory concentration (MIC) of > = 22mm was interpreted as methicillin-resistant (MRSA) while MIC of < =21 was interpreted as methicillin-susceptible (MSSA).

Results: A total of 418 nonduplicate *S. aureus* isolates were collected between October 2019 and December 2020. Out of these, 239 (146 MRSA and 93 MSSA) *S. aureus* isolates were randomly selected for molecular detection for the presence of *mec*A, *mec*C, and *pvl* genes. MRSA prevalence was 49 % (206/418), while 39 % (93/418) harbored the *pvl* gene. *mec*C gene was not detected. Comparative MRSA detection with cefoxitin disc diffusion test, Vitek-2 system, and GenoType MRSA showed perfect categorical agreement. The highest MRSA prevalence was noted in the pediatrics department at 52.5%, followed by surgical (50%) and medicine departments (49.3%). Less than 20% of the MRSA isolates were resistant to tetracycline, erythromycin, and sulfamethoxazole/trimethoprim. All MRSA isolates were sensitive to teicoplanin, linezolid, and vancomycin.

Conclusions: The prevalence of MRSA in Kenyatta National Hospital is high (49.2%) when weighed against a global prevalence of 29% to 35%. The presence of *pvl*-positive MRSA in the hospital setup is a concern considering it can cause serious disease outbreaks. The Cefoxitin disc test can be used as a cheap alternative for screening MRSA, especially oxacillin-sensitive phenotype. However, creating an algorithm to complement the Vitek-2 system for confirming MRSA can be a valuable addition to ensure accurate identification.

CHAPTER ONE: INTRODUCTION

1.0 Background

Staphylococcus aureus causes various infections such as lower respiratory tract and surgical site infections (1). While it can occur as normal flora in healthy humans, *S. aureus* can also be a major cause of numerous diseases such as infections of skin and soft tissues or in severe cases septicemia and infective endocarditis (2). Working with United States national hospitalizations and resistance data to estimate the annual hospitalizations and deaths, Eili Klein et al reported a 62% increase in incidences of hospitalizations due to *S. aureus*-related diagnoses (3). Most isolates of *S. aureus* are resistant to penicillin (4), in contrast, there are those sensitive to stable penicillin-like oxacillin and methicillin. Methicillin and oxacillin-resistant strains, described as methicillin-resistant *S. aureus* (MRSA), are non-susceptible to all β-lactam agents except fifth-generation cephalosporin like ceftaroline (4). The resistance of *S. aureus*, especially MRSA against antimicrobial agents has widened to include quinolones, aminoglycosides, and macrolides (5,6,7,8). The use of macrolides (e.g., erythromycin) as an alternative drug for MRSA has been hampered by the development of macrolide resistance (9). Moreover, resistance to lincosamides like clindamycin, commonly preferred for the treatment of skin and soft tissue infections caused by *S. aureus* has also been detected (10,11). Lack of susceptibility to penicillin, cephalosporin, and related antibiotics in *S. aureus* is induced by the production of the enzyme β-lactamase (12). The production of penicillin-binding protein 2a (PBP 2a) with reduced attraction for β-lactam antibiotics is the second mechanism of resistance (13). Expression of *the mec*A gene encodes for methicillin resistance (14). Mechanisms of oxacillin resistance other than *mec*A cannot be completely ruled out (15).

MRSA detection is complicated by many factors for example, methicillin resistance is diverse in the majority of *S. aureus* isolates with strains expressing heteroresistance (16) and others showing borderline resistance (15). Consequently, laboratories come up with techniques that increase the expression of resistance in Staphylococci, such as supplementation of media with Sodium chloride and extending the incubation period to 48 hours (17). Conventional methods of antibiotic susceptibility testing including disk diffusion or broth micro-dilution take long to perform requiring a minimum of 24 hours to obtain results. Furthermore, the identification of MRSA may be affected by minimal expression of resistance to oxacillin by specific *S. aureus* strains (18). It may not be easy to differentiate MRSA from borderline oxacillin-resistant *S. aureus* (18). Generally, identification of MRSA by molecular methods is only possible in reference laboratories since clinical laboratories do not usually perform PCR or DNA

hybridization for *the mec*A gene. When standard protocols are followed, conventional microbiology tests can be used to detect MRSA (19). The cefoxitin disk diffusion method can be used to complement routine test methods or as an alternative test (20). Molecular tests, like the polymerase chain reaction (PCR), could be used to directly detect *mec*A (21).

1.1 Statement of the Problem

MRSA is associated with high mortality and morbidity in hospitals globally (22). Accurate diagnosis is critical for prompt management of cases, infection control, and reduction of costs resulting from prolonged hospital stays and unnecessary use of antibiotics. Recent studies (23, 24) have reported varying MRSA prevalence of between 3.7% and 84% making it difficult to tell the truth from exaggerated MRSA prevalence in hospitals around Nairobi. There are many challenges in the detection of MRSA; some result from the heterogeneous nature of Methicillin resistance in *S. aureus* (15), and others due to quality and methodological issues (25). We do not know which of these factors is responsible for variance in the reported prevalence.

1.2 Justification of the Study

There is great variation in reported MRSA prevalence in hospitals within Nairobi, which has been attributed to the misidentification of coagulase-negative Staphylococci as *S. aureus* (23). There is not a single technique for the detection of MRSA that is 100% sensitive (26), hence the need to use a testing algorithm that combines manual phenotypic methods such as cefoxitin disc diffusion with either automated or molecular approaches. The detection of *mecA* by PCR has been hailed as the gold standard for the detection of MRSA (25). However, the discovery of *mecC,* a divergent *mecA* homologue that also codes for methicillin resistance, has necessitated the use of kits that screen for both *mecA* and *mecC*. GenoType MRSA^{TM} molecular method is a multiplex PCR technique that detects not only *mecA*, *mec*C, and *pvl* genes but also has genes specific for *S. aureus* and *S. epidermidis* making it ideal for validating phenotypic methods for detecting MRSA with a high degree of accuracy. This study aimed to compare the diagnostic accuracy of the Vitek 2 system (bioMérieux, La Balme les Grottes, France) with cefoxitin disc diffusion methods for detecting MRSA prevalence at KNH and evaluating areas for improvement.

1.3 Study Objectives

1. 3.1 General Objective

To compare the diagnostic accuracy of the Vitek-2 system and cefoxitin disc-diffusion tests in detecting the prevalence of MRSA in clinical samples at Kenyatta National Hospital, with Genotype MRSA PCR serving as the gold standard. In addition, describe the antibiotic resistance pattern of MRSA and their distribution within the hospital departments.

1.3.2 Specific Objectives

1. To use both the Vitek-2 system and cefoxitin disc-diffusion test to determine the proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical samples from KNH patients.
2. To determine the correlation of Genotype MRSA PCR with Vitek-2 and cefoxitin in detecting MRSA.
3. To describe the frequency distribution of MRSA isolates by hospital departments
4. To describe the resistance pattern of MRSA isolates to various antibiotics.

CHAPTER TWO: LITERATURE REVIEW

2.1 Burden of MRSA

Methicillin-resistant *S. aureus* (MRSA) is frequently encountered in healthcare facilities worldwide (22). It accounts for 13-74% of all clinical *S. aureus* isolates (26, 27, 28). There is much global and regional variation in the epidemiology of MRSA (29). Greece, Spain, Italy, Israel, and Croatia as well as other European countries taking part in the regional Antibiotic Resistance Surveillance (EARSS) have all reported a prevalence of at least 25% from blood cultures (30). Countries in Asia, Malta, and North and South America have reported rates above 50% (22, 28). In the first WHO report on AMR, a huge difference in surveillance data was noticed in Africa and developing countries unlike in Western countries where records on the burden of MRSA do exist (31). Research has shown differences in reported MRSA prevalence both within-country and across countries with prevalence rates ranging from 21-47% reported in Cameroon, Uganda, and Ethiopia (19, 20, 30). Additionally, a prevalence of below 10% has been reported in Tunisia, Malta, Algeria, and Eritrea (31, 32). Separate relevant studies on MRSA give variable findings making it difficult to generalize relevant categorical conclusions (33). Omuse et al in a study of *S. aureus* isolate in a private referral hospital in Nairobi reported an MRSA prevalence of 3.7% for the year 2011 to 2013 as determined by the Vitek 2 system (23). Maina et al, 2012, investigating staphylococcal infections of the skin and soft tissues in five public hospitals in Nairobi reported an MRSA prevalence of 84.1% (24).

2.2 Molecular Mechanisms of MRSA

MRSA results from the transformation of methicillin-susceptible *S. aureus* (MSSA) when the Staphylococcal cassette chromosome *mec* (SCC*mec*) is inserted into the chromosome gene *orf*X of the Staphylococcal cassette *mec* (SCC*mec*) (35). SCCmec is a mobile genetic element, bearing the only mediator for methicillin resistance, named *mecA* or *mecC* gene (36). Acquisition of *mec*A makes MRSA resistant to β-lactams and other treatment options are required in infections due to this group of strains (36) MSSA acquired methicillin resistance gene, *mec*A or *mecC*, through horizontal gene transfer *mecA* gene encodes a modified trans-peptidase, known as penicillin-binding protein PBP2a with a lower affinity for beta-lactam antibiotics thus conferring methicillin resistance (36,14). Trans-peptidases are involved in cell wall synthesis (37). Other genes identified through troposome mutagenesis as contributing to cell wall synthesis include *fem*A and *fem*B which play a role in pentaglycine side chain formation in the characteristic cross-linked peptidoglycan cell wall of *S. aureus* (38).

Resistance against multiple drugs (MDR) is where organisms acquire resistance to numerous chemotherapeutic agents (39). Penicillin resistance is encoded by R plasmid via the *blaZ* gene carrying the enzyme penicillinase which is responsible for the inactivation of penicillin through the splitting of the beta-lactam ring (40). Resistance against quinolones is by point mutations at the topoisomerase subunits at the Gr1A site of topoisomerase IV as well as in the Gyrase subunit GyrA or by overexpression of efflux pumps (39). Genes labeled *rpoB, vraS, and msrR* have mutations responsible for vancomycin resistance (39, 40).

2.3 Hospital-acquired versus community-acquired MRSA

Health-care-associated MRSA (HA-MRSA) serotypes cause nosocomial infections and belong to SCC*mec* type I, II, or III (43). There has been an evolution in the study of transmission and control of MRSA in the past following the appearance of community-acquired MRSA (CA-MRSA) (3). Upon importation into hospitals, CA-MRSA can cause nosocomial infections (3), leading to distortion of the epidemiological definition of CA-MRSA (3). CA-MRSA causes a variety of morbidities like skin and soft tissue infections (SSTIs), bloodstream, bone, severe pneumonia, and fasciitis in the military, children, sportsmen, or immunosuppressed individuals. CA-MRSA usually possesses Panton-Valentine Leucocidin (*pvl*) genes together with SCC*mec* IV or V (42, 43). Panton valentine leucocidin comprises of two-part cytotoxin that can perforate and kill leucocytes (46) contributing to the extreme harmfulness of CA-MRSA (47) and can cause the expression of other virulence factors (48) such as inflammatory responses due to the toxin.

2.4 MRSA in KNH

Kenyatta National Hospital acquired the Vitek 2 system in mid-September 2013. Studies on *S. aureus* conducted before then relied on conventional microbiological techniques to identify bacteria such as gram stain, catalase test, hemolysis on blood agar, and tube coagulase test. Kirby-Bauer's disc diffusion method was used to carry out AMR tests. The challenges with these methods were many such as the effects of incubation temperature, media pH, differences in individual judgment on zone sizes, quality of sensitivity disc due to storage conditions, and many more. It is against such background that Rutare et. al., reported an MRSA prevalence of 46.5% (49) among pediatric patients in intensive care at the hospital compared to the prevalence of between 25% to 35% reported from subsequent studies using the Vitek 2 system (48,49). Latex agglutination tests based on specific monoclonal antibodies targeting PBP2a antigen can also be used to detect MRSA (52). In addition, CHROMagar which makes use of coloring agents in the medium to identify MRSA is used by some laboratories (53).

Lack of standard inter-laboratory testing and identification of isolates can affect MRSA reports where phenotypic methods show higher rates than molecular approaches (34). Erroneous reporting of Staphylococci which are coagulase-negative (CONS) as *S. aureus* may lead to overestimation of methicillin resistance (23). The contention on the accuracy of MRSA detection and subsequent reports on prevalence may be resolved by the use of a combination of manual conventional methods and PCR molecular assay (50). There is limited MRSA data in most African countries due to inadequately equipped healthcare facilities, few trained professionals, and lack of active surveillance (32, 52).

2.5 Methodological approaches in the detection of MRSA

Some of the accomplished microbiological methods of detecting MRSA include oxacillin microdilution and oxacillin screen agar (55). The Clinical Laboratory Standards Institute (CLSI) recommends the cefoxitin disc diffusion method for the detection of MRSA (56). Oxacillin disc method fails to detect heterogeneous MRSA hence not recommended (55). For quick detection of MRSA, real-time PCR technology has been used instead of culture methods (21). *Mec*A gene is highly conserved among Staphylococci, thus making its detection by PCR be regarded as the gold standard for the identification of MRSA (57). Conversely, PCR being costly and requiring trained skills, it is mostly used in reference centers (57). However, even with these advanced methods, there have been reports of erroneous identification of *S. aureus* with the use of both chromogenic agar plates (58) and PCR methods (59).

2.5. 1 Cefoxitin disc diffusion

Cefoxitin belongs to the cephalosporin class of antibiotics that induces PBP (2a) which codes for the *mec*A gene (57). Cefoxitin has been reported to be a better inducer of *the mec*A gene than penicillin (58). Some studies have reported sensitivities of between 94% to 98% and specificities of 95% to 100% respectively for the cefoxitin disc diffusion method to detect MRSA (20, 58). To increase the detection of hetero-resistant strains of *S. aureus*, the following procedures are advised: (i) incubating AST plates at 30 to 35^{o} C, (ii) utilization of media comprising of 5% sodium chloride; (iii) use of a heavier inoculum such as 0.60 instead of routine 0.5 McFarland as well as (iv) prolonging incubation time to 48h (59,60, 61). Disc diffusion tests have been shown to reliably detect MRSA when performed at 30 to 35^{o} C (61, 62).

2.5.2 Vitek-2 system

Automated systems are preferred for giving results in a few hours though most of them have disappointingly low sensitivity more so for heterogeneous resistance (57, 64). However, they have high specificity (66). The accuracy of the Vitek 2 system (bioMérieux, France) to correctly identify *S. aureus* species is documented to be between 95 and 99% (60), 98.3% categorical agreement for testing Staphylococcus compared with a combination of phenotypic and genotypic tests (67) and low false positive rates approaching 1.1% (68). However, the sensitivity of the Vitek 2 system to detect low-level oxacillin MRSA has been reported to range from 69.6 % to 84.5% (69).

2.5.3 Geno Type MRSA test

GenoType^{R} MRSA (Hain Life Science Nehren, Germany) test is a nucleic acid amplification assay that detects the presence of *mecA* and, *mecC* which confer methicillin resistance as well as *pvl* gene that codes for two-component cytotoxin virulence factor, panton valentine leucocidin (70). The isolates are only considered MRSA positive when both *nuc* gene specific to *S. aureus* and *mecA* or *mecC* genes are detected (71). The kit also detects *S. epidermidis* and controls the contamination with coagulase-negative staphylococci. The stated sensitivity and specificity is 100% (72).

2.6 *PVL* and MRSA

Panton-Valentine Leucocidin (*pvl)* is an exotoxin produced by some strains of *S. aureus*. It consists of two component proteins Luk F-Pv and Luk S-Pv that target monocytes, macrophages, and neutrophils killing them by apoptosis (70, 71). *Pvl*-producing *S. aureus* causes severe and recurrent skin and soft tissue infections. Several studies have suggested that *pvl* could be used as a marker for CA-MRSA (72,73, 74). Unlike HA-MRSA which carries Staphylococcal cassette *mec* type I-III, CA-MRSA carries the shorter Staphylococcal cassette *mec* type IV and V (SCC*mec* IV, V) that confers only low-level methicillin resistance and lacks additional resistance genes (77). However, studies from West and Central Africa have shown at least 40% of methicillin-susceptible *S. aureus* (MSSA) from these regions to be *pvl*-positive (75, 76).

The prevalence of *pvl* from clinical *S. aureus* isolates varies from country to country ranging from a low of 9.7% in England to a high of 57% in West Africa (76, 77). In Kenya, Omuse et. al., working on *S. aureus* isolates from a hospital in Nairobi reported a prevalence of 39.7% (81).

2.7 Treatment of MRSA

The majority of MRSA isolates are susceptible to glycopeptide agents; vancomycin, teicoplanin, quinupristin-dalfopristin, and linezolid (82) and are therefore frequently used to treat hospitalized patients with complicated soft tissue infections (83). Empiric oral treatment for staphylococcal skin and soft tissue infections (SSTI) include trimethoprim/ sulfamethoxazole, clindamycin, doxycycline, and linezolid especially those caused by susceptible CA-MRSA (84). The standard treatment regime for bacteremia recommended by the Infectious Diseases Society of America (IDSA) is vancomycin or daptomycin with ceftaroline as the alternative (85). The treatment of HA-MRSA isolates is complicated since most are multidrug-resistant thus reducing the choice of drugs available for use (86). The presence of low-level methicillin-resistant *S. aureus* that is often misidentified as MSSA further complicates their treatment with beta-lactam-based antibiotics to which they are resistant (69).

2.7.1 Control of MRSA

Successful implementation of infection control and preventive measures like carrying out MRSA risk assessment, and ensuring proper hand hygiene (83, 84) is recommended in hospital settings. Control of the source of infection includes daily bathing of patients at risk of MRSA with chlorhexidine gluconate reportedly reduces the spread of potential pathogens (89). Use of standard precautions such as barrier methods like gloves, face masks, and gowns when handling skin and soft tissue infected patients is advised to prevent the spread (90).

2.8 Conceptual framework

Studies on MRSA prevalence conducted in Kenyatta National Hospital around Nairobi and elsewhere in Kenya have reported very varied results so it is difficult to tell the true prevalence. To obtain the maximum sample size, we used a conservative prevalence of 50%. All *S. aureus* isolates identified as MRSA by Vitek2 were retested with cefoxitin disc diffusion method and confirmed by molecular method. At the same time, 93 of MSSA were randomly selected and tested by molecular method for comparative purposes.

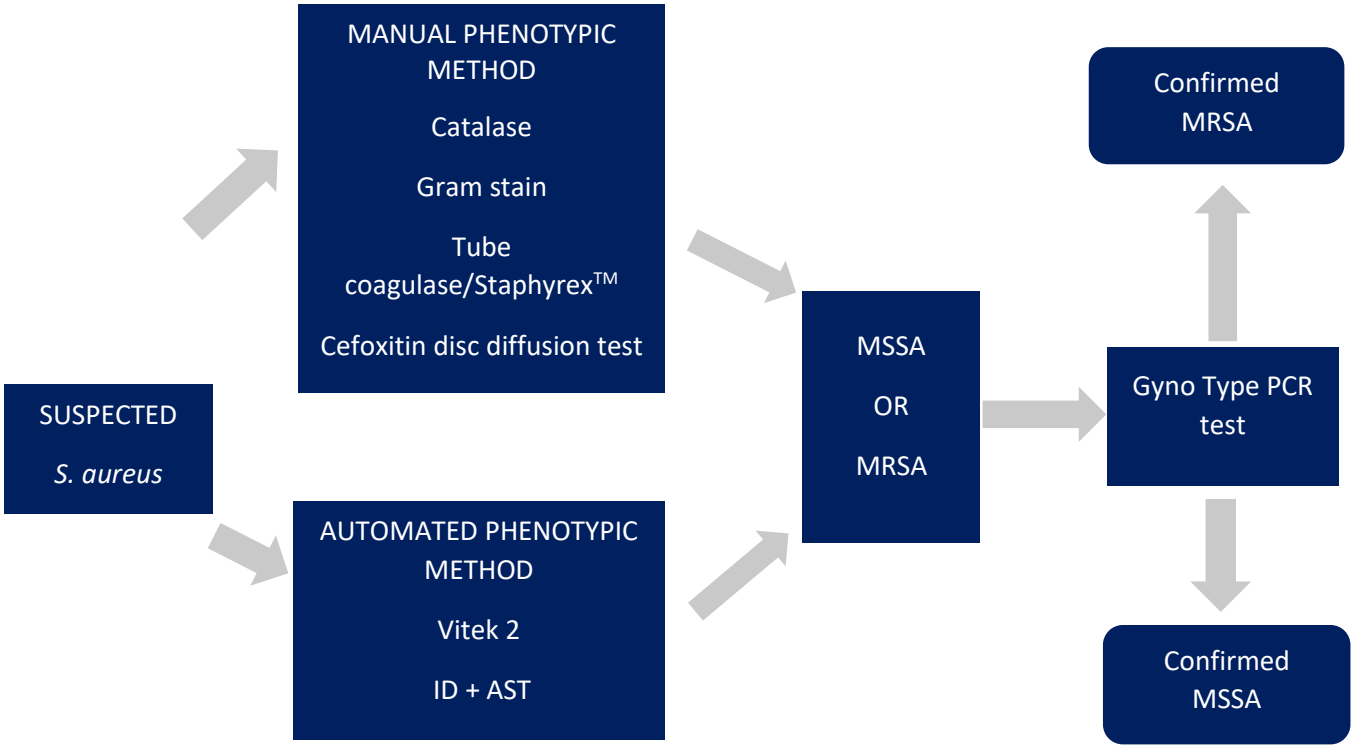


Figure 1: Schematic presentation of the conceptual framework

CHAPTER THREE: METHODS

3.1 Study Design: This was a cross-sectional analytic study design.

3.2 Study Area: This study was conducted at Kenyatta National Hospital, a level 6 public hospital under Kenya's Ministry of Health. It is the largest public referral hospital in Kenya. The KNH microbiology laboratory processes an average of 16,000 cultures from various specimens per year. The choice of KNH was informed by the diversity of its patients nearly represent the Kenyan population. Still, the variation in the few published studies on MRSA at KNH necessitated a more comprehensive study.

3.3 Study Population: All patients whose specimen cultures at KNH microbiology yielded *S. aureus* during the time of the study.

3.3.1 Inclusion criteria: All non-duplicate clinical specimens with requisitions for culture and sensitivity that were received at KNH microbiology laboratory and yielded *S. aureus* are considered clinically significant.

3.3.2 Exclusion criteria: *S. aureus* isolates from hospital surveillance samples.

3.4 Sample size: The sample size was computed according to the formula given by Lemeshow et al in 1986, for calculating sample size in health studies (91):

Sample size = $Z^2 * (p) * (1-p) / C^2 = (1.96)^2$ 0.5*0.5/0.0025= 385

Z = Z value for 95% confidence interval.

P = population proportion, taking worst case scenario as (0.5)

C = margin of error = 0.05

3.5 Study Period: *S aureus* isolates obtained from October 2019 to December 2020

3.6 Sampling Method: All clinically significant gram-positive cocci that were catalase-positive from various specimens were identified by the Vitek-2 system. They were then sub-cultured on blood agar plates to ensure they were pure culture and were classified as either MRSA or MSSA as per their initial interpretation of the cefoxitin screen test and oxacillin minimum inhibitory concentration based on CLSI recommendations. They were retested for susceptibility to cefoxitin disc (30μg) on Mueller Hinton agar, a zone of inhibition <= 21mm was interpreted as resistant and those above 22mm as susceptible. They were then stocked at -70^o C to await PCR. The PCR samples were selected randomly with a target of 250 limitations

based on available kits. Every 3/5 (250/418) samples were selected such that samples were arranged randomly in one file and starting from one end, a count of one to five was done. Every first, third, and fifth sample was then selected from the Vitek-2 system grouped MRSA and MSSA.

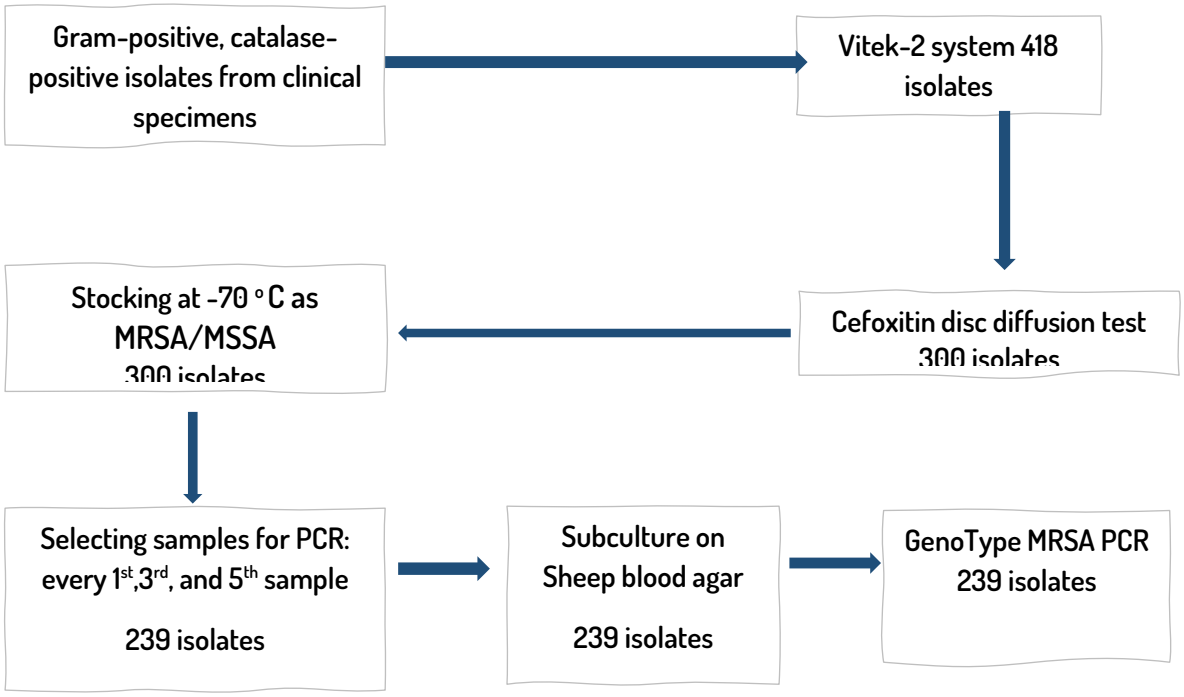


Figure 2: Schematic Representation of the Laboratory procedures

3.6. 1 Laboratory Methods

3.6.2 Isolation, identification, and susceptibility testing of *S. aureus*

All suspected *S. aureus* isolated from urine, sputum, blood cultures, pus swabs, and aspirates were identified using standard microbiology procedures namely gram stain, hemolysis on blood agar, catalase, and coagulase. In parallel all gram-positive, catalase-positive isolates were identified using the Vitek-2 system (bioMérieux, France) gram-positive card. Antibiotic susceptibility testing was performed using the Vitek 2 system and P580 card comprising of the following antibiotics: vancomycin, teicoplanin, tetracycline, linezolid, tobramycin, gentamycin, levofloxacin, moxifloxacin, erythromycin, trimethoprim /sulfamethoxazole, and penicillin. Isolate purity was ascertained by subcultures while confirmation of *S. aureus* was done using Staphyrex^{R} (Remel, UK) latex agglutination test. Using CLSI guidelines (56), *S. aureus* isolates that showed resistance to cefoxitin or oxacillin were documented as MRSA.

MRSA isolates were then confirmed by the cefoxitin disc diffusion method and molecular detection of *the mec*A gene.

3.6. 3 Detection of MRSA by Cefoxitin disc test

All non-duplicate *S. aureus*, identified through the Vitek-2 system in 3.6.2 above, were streaked onto sheep blood agar, gram-stained, and checked for hemolysis. For comparative purposes, susceptibility to cefoxitin 30 µg (Oxoid, England) disc was performed using the Kirby-Bauer disc diffusion method as described by Amita Jain et al (20). Zones of inhibition in millimeters were interpreted according to CLSI guidelines where <=21mm was interpreted as resistant while >=22 was interpreted as susceptible (83). The isolates were preserved in skimmed milk at -70^{o} C until they were tested for *mecA* genes.

3.6. 4 PCR detection of *mec*A, *mecC,* and *pvl* genes

Out of the 418 isolates, 239 were randomly selected, where the isolates were mixed and arranged linearly. For every bunch of five isolates, the first, the third, and the fifth were picked until a count of 239 was obtained. Six tests were also dedicated to positive and negative controls.

Simultaneous detection of *mecA*, *mecC,* and *pvl* genes was done using GenoType MRSA® version 3 kit (Hain Life Science, Nehren, Germany) following the manufacturers' recommendations (72) and utilized as per the method described by Otte et. al., (70). Briefly, the Genotype MRSA test is based on the nitrocellulose DNA strip technology, a type of nucleic acid lateral flow immune assay (NALIA) that detects DNA using capture and labeled reporter antibodies (streptavidin) (92). The protocol involved the following steps: (i) DNA was extracted from *S. aureus* cultures, where 5 colonies from a pure culture were collected with an inoculation loop and suspended in 150µl of molecular grade water, ensuring that no culture medium was transferred. The bacterial suspension was incubated at 95^{o}C for 15 minutes in a heating block followed by incubation in an ultrasonic bath. It was then spun at a speed of 14,000 g on a tabletop centrifuge for 5 minutes and directly used for PCR. (ii) Multiplex amplification with biotinylated primers and polymerase was added in the Amplification Mixes AM-A and AM-B and optimized for this test. After thawing, AM-A and AM-B were spun briefly after mixing carefully. Pipetting AM-A and AM-B were only done in a separate room free from contaminating DNA. The Hot Star Taq DNA polymerase supplied together with other reagents in the kit, was added in a separate working area. To prepare each sample, 10 µl of AM-A and 35 µl of AM- B were pipetted, followed by 5µl of DNA solution to make a final volume of

50µl. The PCR cycling conditions consisted of an initial denaturation step of 15 minutes at 95 ^{o} C for one cycle followed by repeating cycles of denaturation (20 seconds at 95 ^{o} C) and annealing (30 seconds at 60^{o}C) for 22 cycles.

iii) A reverse hybridization of the DNA STRIP with the kit reagents and a twin incubator machine as recommended by the manufacturer.

3.7. Internal quality control

All procedures were performed with strict compliance with prescribed standard operating procedures (SOPs). In all the assays performed, MRSA ATCC 43300 was used as a positive control while methicillin-susceptible *S. aureus* (MSSA) ATCC 29213 was used as a negative control.

3.8 Data management

3.8.1 Data entry

Data on antibiotic sensitivity testing obtained from the Vitek-2 database was entered into WHONET software where it was analyzed and presented in tables and charts. Qualitative data on cefoxitin resistance by manual methods, presence or absence of *mecA*, *mecC*, or *pvl* genes were entered onto an Excel worksheet containing specimen number, specimen type, and location of the patient (see appendix **6.3**). Both hard copies and soft copies of test results were maintained for verification until the completion of analysis.

3.8.2 Data cleaning, storage, and security

All the data was verified for accuracy using original data sources e.g., Vitek 2 data files and worksheets before and after transferring to data collection tools to eliminate any transcription errors. Original hard copies of results were filed in a box file and secured in a lockable cabinet for safety. Soft copies of results were saved in a secured personal computer until after publication of the final report.

3.8.3 Data analysis

Antibiotic sensitivity test data from Vitek 2 covering the study period was downloaded and imported to WHONET software, analyzed, and presented in the form of tables and charts showing the percentage of susceptible and resistant *S. aureus* isolates. Qualitative data from molecular testing was captured in template tables showing the presence or absence of *mec*A, *mec*C, and *pvl* genes. This PCR data was entered into the Excel sheets containing data from Vitek 2, analyzed by Excel, and presented in the form of two-by-two tables used for

computation of relative agreement between the methods by the Cohen kappa correlation coefficient. Sensitivity, specificity, positive and negative predictive values were calculated using a contingency table as shown below:

	Comparative test results		
Candidate test results	Positive	Negative	Total
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	*n*

a: positive results concordance; b: negative results disagreement; c: positive results disagreement; d: negative results concordance; *n*: total number of samples

PC[%]=a/(a+c)x100; NC[%]=d/(d+b)x100;OC[%]=(a+d)/*n*x100

3.9 Study results dissemination

Study results will be presented to the KNH antimicrobial Stewardship subcommittee, KNH Research and Programs department, scientific workshops and conferences of health professionals, as well as departmental meetings. A copy of the study report will also be submitted to the KNH /UON ethics and research committee before publication in a medical journal.

3.10 Ethical considerations

This was a laboratory-based study without direct involvement of patients (subjects). However, this study was approved by the KNH/UON Ethics & Research Committee and registered as P355/07/2020 as shown in Appendix 6.5. Requesting clinicians were promptly notified of study subjects with MRSA for immediate intervention to prevent spread. Laboratory results used for this study bore no patient identifiers such as name, inpatient (IP) or outpatient (OP) number, and laboratory number after the data clean-up exercise. However, to meet objective 3, patient hospital locations were used in the data analysis.

CHAPTER FOUR: RESULTS

4.1 Study *S. aureus* isolates

A total of 418 non-duplicate *S. aureus* isolates were obtained for the period between October 2019 and December 2020. These isolates were sourced from various specimen types as shown in table 1 below. The majority (56.4%, n=236) of *S. aureus* were isolated from pus specimens, followed by blood at 19.3% and tracheal aspirates at 15.5%. The locations with the highest isolation rates for *S. aureus* were the surgical department which contributed 34.9% (146/418) of all study isolates, followed by medicine at 22.2% (n=116).

Table 1. Distribution of *S. aureus* by specimen types from various hospital departments at Kenyatta National Hospital

	Specimen Types				
Location	**Pus**	**Blood**	**Tracheal aspirates**	**Other Specimens**	**Totals isolates**
Pediatrics & Newborn Unit	14	19	1	6	40
Critical care Unit	9	11	62	2	84
Surgical	126	7	1	12	146
Medicine	51	29	2	12	93
Burns unit	22	8	1	1	32
Other locations	13	7	0	3	23
TOTAL	**236**	**81**	**65**	**36**	**418**

4.2 Correlations of the methods

Of the 418 *Staphylococcus aureus* isolates identified by Vitek 2, 201 were MRSA while 217 were MSSA. This classification was based on CLSI. Fifty-one out of 201 (25%) cefoxitin-positive *S. aureus* isolates had oxacillin MIC of less than 2 mg. Two hundred and thirty-nine (146 MRSA and 93 MSSA) *S. aureus* were selected for multiplex PCR assay to determine the presence/absence of *mec*A, *mec*C, or *pvl* genes. No *mecC* gene was detected. One hundred and forty-one were MRSA**,** while 98 were MSSA. The prevalence of MRSA for the PCR sample was 59%, while that of MSSA was 41%.as shown in Figure 3 below.

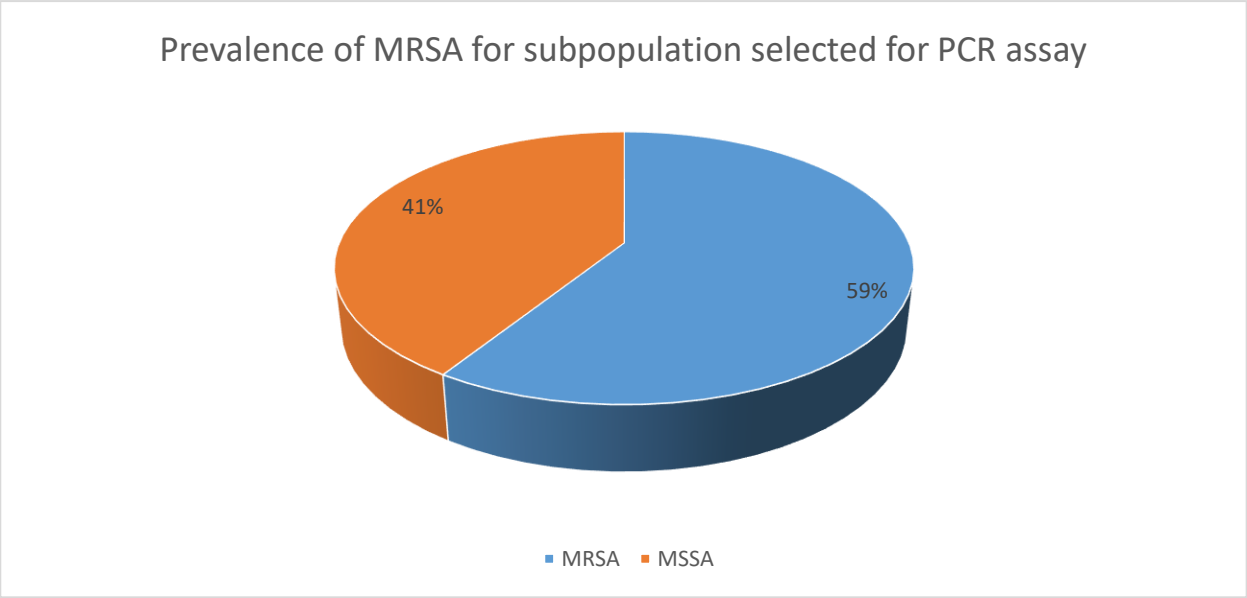


Figure 3. Prevalence of MRSA by GenoType MRSA™ PCR

The results for the Cefoxitin and Vitek-2 system were entered into a two-by-two table and compared with those from Genotype MRSA PCR. The results were as shown in table 2 below:

Table 2. Correlation of Vitek-2 with GenoType MRSA in Detecting MRSA

Test	**GenoType MRSA**			
		MRSA	MSSA	Total
Vitek-2 system	MRSA	141	0	141
	MSSA	0	98	98
	Total	141	98	239

Positive predictive value for Vitek 2 system = TP/TP+FP=141/141=100%, Sensitivity=TP/(TP+FN) = (141/141) =100%, Negative predictive value = TN/TN+FN=98/98=100%, Positive predictive value = TP/TP+FP*100=141/141*100=100%, where TP=total positive, FP=False positive, FN=false negative, TN=Total negative

Positive predictive value, negative predictive value, sensitivity, specificity, and Cohen kappa categorical agreement for Vitek 2 and Cefoxitin disc test were calculated. The results were as shown in the table 3 below:

Table 3. Correlation of Cefoxitin disc-diffusion test with GenoType MRSA in detecting MRSA

		GenoType MRSA		
		MRSA	MSSA	Total
Cefoxitin disc-diffusion test	MRSA	141	0	141
	MSSA	0	98	98
	Total	141	98	239

Positive predictive value for cefoxitin disc diffusion test = TP/TP+FP=141/141=100%, Sensitivity=TP/(TP+FN) = (141/141) =100%, Negative predictive value = TN/TN+FN=98/98=100%, Positive predictive value = TP/TP+FP*100=141/141*100=100%, where TP=total positive, FP=False positive, FN=false negative, TN=Total negative

The relative agreement between the Vitek-2 system and the cefoxitin disc-diffusion test by Cohen's kappa correlation coefficient was 1 representing perfect agreement while sensitivity, specificity, and positive and negative predictive values were 100% as shown in table 4 below.

Table 4. Comparison data for Vitek-2 system and Cefoxitin disc-diffusion test in the detection of MRSA

Method	**Detected as MRSA**	**Detected as MSSA**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Concordance with PCR**	**Kappa**
Vitek-2 system	206	212	100%	100%	100%	100%	100%	1
Cefoxitin disc-diffusion test	206	212	100%	100%	100%	100%	100%	1

Since the three methods had a perfect categorical agreement, those isolates in the initial Vitek-2 system-generated data that had the *mec*A gene were interpreted as MRSA. Similarly, those whose cefoxitin disc-diffusion test had a zone of inhibition below 21mm were also reported as MRSA. The overall effect was that 206 isolates out of 418 (49%) were reported as MRSA while 212 (51%) were MSSA as shown in Figure 4 below. The Overall prevalence of MRSA at Kenyatta National Hospital was 49%.

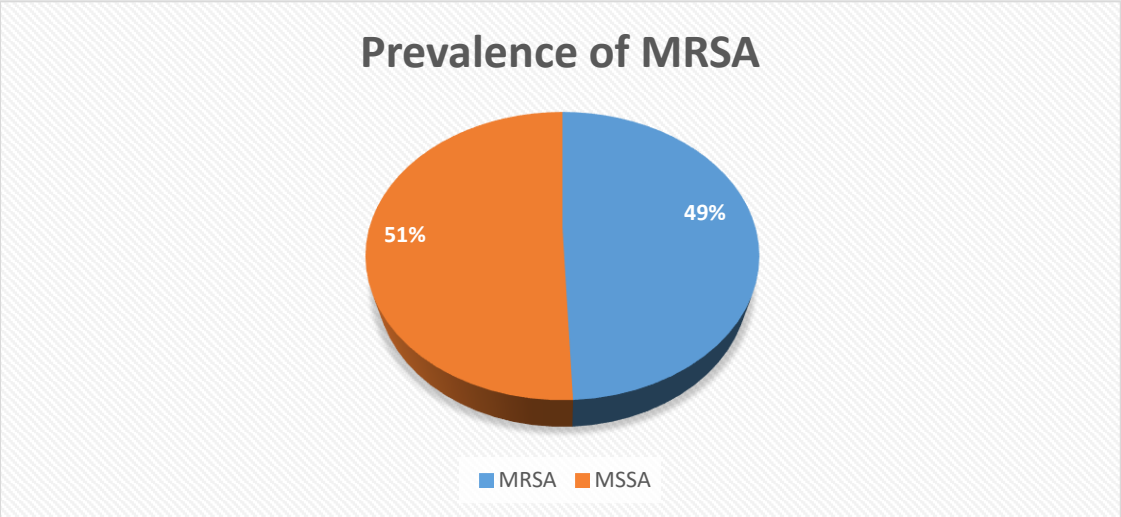


Figure 4: Prevalence of MRSA and MSSA

4.3 Distribution of MRSA by Kenyatta Hospital locations

After validation of Vitek-2 data with the results of GenoType MRSA PCR and cefoxitin disc diffusion tests MRSA and MSSA were classified according to the patient hospital locations and the type of specimen. MRSA was most prevalent in the burns unit at 55.9% (19/34) followed by the pediatric department and critical care unit with prevalence of 52.5% and 48.8% respectively. The distributions of MRSA and MSSA were as shown in the table 5 below:

Table 5. Distribution of MRSA by Kenyatta National Hospital locations and specimen types

	Specimen Types				MRSAMSSA		
Location	**Pus**	**Blood**	**Tracheal aspirates**	**Other Specimens**	**Total MRSA(%)**	**Total MSSA**	**Totals isolates**
Burns Unit	10	7	1	1	19 (55.9%)	15 (44.9%)	34
Pediatrics & Newborn Unit	9	9	1	2	21 (52.5%)	19 (47.5%)	40
Critical care unit	6	8	25	1	40 (48.8%)	42 (51.2%)	82
Surgical	63	3	1	5	72 (49.3%)	74 (50.7%)	146
Medicine	21	17	0	6	44 (47.3%)	49 (52.7%)	93
Other locations	7	2	0	1	10 (43.5)	13 (56.5%)	23
TOTAL	**116**	**46**	**28**	**16**	**206**	**212**	**418**

Pvl gene was detected in 93 of the 239 (39%) *Staphylococcus aureus* isolates tested with the surgical department showing the highest frequency at 41.9% followed by critical care and burns units with 18.3%. Fifty-eight of the 93 (62.4%) *pvl* positive were MRSA while 35 were MSSA. Pus specimens had the highest proportion of all-positive *S. aureus* isolates at 58% (54/93) followed by blood culture specimens at 23.6% (n=22) as shown in table 6 below.

Table 6. Distribution of *pvl* gene by hospital locations and specimen types

	Specimen Types				Pvl %	
Location	**Pus**	**Blood**	**Tracheal aspirates**	**Other Specimens**	**% pvl positive MRSA**	**Total Pvl positive**
Surgical	33	3	0	3	41.9%	39
Critical care Unit	4	4	8	1	18.3%	17
Medicine	7	7	0	2	17.2%	16
Pediatric and newborn unit	3	3	1	1	8.6%	8
Burns	4	2	0	0	6.4%	6
Other locations (Renal, Ear, Nose & throat, etc.)	3	3	0	1	7.5%	7

4.4 Antibiotic Susceptibility Profile of MRSA Isolates

All 141 MRSA isolates were sensitive to teicoplanin, linezolid, and vancomycin while sensitivity to clindamycin and gentamycin was 90.1%. The highest resistance was observed with penicillin where 97.9% of MRSA isolates were resistant followed by /sulfamethoxazole at 62%. Inducible clindamycin resistance was noted in 14 of the 141 isolates (9.9 %). Less than 20% of the isolates were multi-drug resistant. The results are shown in table 7 below.

Table 7. The antibiotic Susceptibility profile of all *S. aureus* isolates and the confirmed MRSA isolates

	ALL ISOLATES (n=418)		MRSA (n=141)	
Antibiotic	**Resistant %**	**Susceptible %**	**Resistant %**	**Susceptible %**
Linezolid	0	100	0	100
Teicoplanin	0	100	0	100
Vancomycin	0	100	0	100
Gentamycin	7.2	92.8	8.5	91.5
Clindamycin	8.6	91.4	9.2	91.8
Levofloxacin	16.3	83.7	19.9.3	81.1
Tetracycline	27.8	72.2	20.6	79.4
Erythromycin	34.9	65.1	38.3	61.7
Trimethoprim/Sulfamethoxazole	64.1	35.9	61.7	38.3
Penicillin	94.7	5.3	97.9	2.1

CHAPTER FIVE: DISCUSSION, ONCLUSION AND RECOMMENDATIONS

5.0 Discussion

The prevalence of MRSA at Kenyatta National Hospital was 49.2% based on the three methods and this was statistically significant p-value =0.003 (X^2 =83). The MRSA prevalence was lower than 57% reported by Kejela et al while studying patients at a referral hospital in southwestern Ethiopia in the year 2022 (93). This was however consistent with the prevalence of below 50% reported by Abubakar et al in a review of studies covering various regions of Nigeria in the year 2018 (94) but higher than that of 25% reported by Stefani et al for countries in Europe (30). These differences could be due to many factors such as the nature of patients being treated, whether there was an ongoing outbreak during the time of the study, effective antibiotic prescribing practices, and antibiotic stewardship implementation. This study also found high MRSA prevalence of 52.5 %, 50%, and 49.3% in the pediatric, critical care unit, and surgical departments respectively. This could have been caused by an ongoing outbreak at the time of study or ineffective infection prevention and control (IPC) measures among healthcare workers such as poor hand hygiene and improper use of barrier methods like the use of gloves.

The results of *mec*A detection agreed with those of the cefoxitin disc-diffusion test and Vitek-2 system and hence Cohen's kappa inter-assay agreement was perfect. The sensitivity, specificity, positive and negative predictive values were 100%. This was comparable to the results of a similar study by Sakoulas et al of a sensitivity of 99.5% and specificity of 97.2% for Vitek 2 reported from a medical center in Israel in the year 2001 (95) and other similar studies (94, 58) that have reported sensitivities above 98%. However, the findings in this study differ from that of Felten et al (97) reported a sensitivity of 92.3% for the Vitek-2 system while working with low-level MRSA. The lower prevalence reported by Felten could be due to heterogeneous resistant MRSA which may be missed in subcultures since only a small subpopulation of the culture bears the resistance gene (98).

Vitek-2 system identifies MRSA based on cefoxitin screen (6µg/ml) and oxacillin (1 mg/ml) broth micro-dilution tests based on CLSI guidelines (56). There are challenges in distinguishing oxacillin susceptible MRSA from borderline methicillin-resistant *S. aureus* (BORSA) caused by the overproduction of penicillinase enzyme (15). Thirty-two oxacillin-sensitive MRSA isolates selected for the PCR assay tested positive for *mec*A confirming they were MRSA. After repeat subcultures, ten *S. aureus* isolates previously detected as MSSA by Vitek 2 system were found to have *mec*A by PCR and were confirmed by both repeat Vitek-2 system and cefoxitin disc-diffusion test. This could be attributed to the emergence of heterogeneous or

low-level (oxacillin-sensitive) MRSA which is misdiagnosed as MSSA by routine phenotypic methods (97). At the same time, five previously detected oxacillin sensitive-MRSA by the Vitek-2 system changed to MSSA with all three methods resulting in a net increase of five MRSA tally in the final Vitek-2 data. The conversion of MRSA to MSSA can also be caused by the use of mixed culture with underlying colonies of enterococci or coagulase-negative staphylococci which may cause false positive MRSA. For this reason, isolate purity should be ascertained by subcultures on blood agar plates before further processing can be done. The 25% of oxacillin-sensitive MRSA was similar to that reported by Witte et al (99).

Antibiotic sensitivity test revealed a common thread between confirmed MRSA and the rest of *S. aureus* isolates. Considering MRSA was resistant to all beta-lactam antibiotics, that meant at least a quarter of *S. aureus* isolates were multi–drug resistant to routinely used antibiotics. All MRSA isolates were sensitive to teicoplanin, linezolid, and vancomycin. The highest resistance was observed with penicillin where 97.9% of MRSA isolates were resistant followed by trimethoprim /sulfamethoxazole at 62%. The results showed that at least 15.7% of MRSA were multidrug-resistant to commonly prescribed antibiotics. This calls for active surveillance to prevent the spread of these strains in the hospital. This study was limited by available resources to the extent that only 239 of the 418 *S. aureus* isolates were tested for *mec*A, *mec*C, and *pvl* genes. To mitigate possible bias, we randomized the sample selection. The study has demonstrated the benefit of a complementary test to Vitek 2 in detecting MRSA by improving the detection from 48% to 49.2%.

Pvl gene was detected in 39% of *S aureus* isolates tested with multiplex PCR. This was comparable to 39.7% reported by Omuse et al (81). However, the 39% *pvl* prevalence contrasts sharply with the 1.6% reported by Mathew J. Ellington et al in 2007 in the United Kingdom (100), where none of the MRSA had pvl genes. The presence of 41.9% *pvl*-positive MRSA in the surgical department contrasted with the 56.8% reported by Bhatta et al in 2016 while working on *S. aureus* isolates from various surgical and critical care units at a hospital in western Nepal (101). The highest proportion of *pvl*-positive isolates was from pus specimens at 58% (n=54) followed by blood at 17.2%. Whereas the presence of *pvl* is a reliable marker of community-associated MRSA (74,100), the presence of *pvl* in MRSA isolates from the ICU department warrants further studies on molecular characterization of the isolates to rule out HA-MRSA harboring the *pvl* gene. *Pvl*-producing *S. aureus* has been associated with severe and recurrent skin and soft tissue infections as well as necrotizing pneumonia which is fatal (102).

5.1 Conclusions

The prevalence of MRSA in Kenyatta National Hospital is high (49.2%) when weighed against a global prevalence of 29% to 35% (26). There is a need for active surveillance to prevent MRSA from spreading within the hospital setup. The emergence of *pvl*-positive MRSA is a cause for concern as it may lead to increased morbidity and mortality. Cefoxitin disc diffusion test can be used as a cheap alternative to PCR to confirm MRSA, especially oxacillin-sensitive phenotype. An algorithm to complement the Vitek-2 system for confirming MRSA including cefoxitin disc diffusion and PCR can be a valuable addition to ensure accurate MRSA identification.

5.2 Recommendations

Considering that the discordant oxacillin sensitive and cefoxitin screen positive results are common, there is a need for a test to complement the Vitek-2 test for MRSA to ensure accurate diagnosis. Such a test can be a latex agglutination test for PBP2a or the cefoxitin disc diffusion test.

Infections caused by *pvl*-bearing *S. aureus* seemed more prevalent than those from *pvl* negative begging the question, are they being imported from the community to the hospital? To answer that question, we recommend screening patients for MRSA on admission.

REFERENCES

1. Richards MJ, Edwards JR, Culver DH. Nosocomial Infections in Pediatric Intensive Care Units in the United States. 1999;103(4):1–7.

2. Wertheim HFL, Melles DC, Vos MC, Leeuwen WV, Belkum AV, Verbrugh HA, et al. Subscription Information : Review The role of nasal carriage in Staphylococcus aureus infections. 2005;5(December):751–62.

3. States U, Klein E, Smith DL, Laxminarayan R. Hospitalizations and Deaths Caused by Methicillin-Resistant Staphylococcus aureus, 2007;13(12):1999–2005.

4. Becker K, Eiff C. Staphylococcus , Micrococcus , and other catalase-positive cocci. In: Manual of Clinical Microbiology. 2011. p. 308–30.

5. Katayama Y, Ito T, Hiramatsu K. Genetic Organization of the Chromosome Region Surrounding mecA in Clinical Staphylococcal Strains : Role of IS 431 - Mediated mecI Deletion in Expression of Resistance Resistant Staphylococcus haemolyticus. 2001;45(7):1955–63.

6. Baddour MM, Abuelkheir MM, Fatani AJ. Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, Saudi Arabia. Ann Clin Microbiol Antimicrob. 2006;

7. Koyama N, Inokoshi J, Tomoda H. Anti-Infectious Agents against MRSA. 2013;204–24.

8. Torimiro N, Aa M, Sa E. Analysis of Beta-lactamase production and Antibiotics resistance in Staphylococcus aureus strains. 2013;5(3):24–8.

9. Leclercq R. Mechanisms of Resistance to Macrolides and Lincosamides : Nature of the Resistance Elements and Their Clinical Implications. 2002;34.

10. Srinivasan A, Dick JD, Perl TM. Vancomycin Resistance in Staphylococci. 2002;15(3):430–8.

11. Johnson AP, Woodford N. Leading articles Glycopeptide-resistant Staphylococcus aureus. 2002;621–3.

12. Richaiond MH. Dominance of the Inducible State in Strains of Staphylococcus aureus Containing Two Distinct Penicillinase Plasmids. 1965;90(2):370–4.

13. Hartman BJ, Tomasz A. Low-Affinity Penicillin-Binding Protein Associated with r-Lactam Resistance in Staphylococcus aureus. 1984;158(2):513–6.

14. Matsuhashi M, Song MD, Ishino F, Wachi M, Doi M, Inoue M, et al. Molecular cloning of the gene of a penicillin-binding protein is supposed to cause high resistance to β-lactam antibiotics in Staphylococcus aureus. J Bacteriol. 1986;167(3):975–80.

15. Hryniewicz MM, Garbacz K. Borderline oxacillin-resistant staphylococcus aureus (BORSA) - a more common problem than expected? J Med Microbiol. 2017;66(10):1367–73.

16. Chambers HF. Methicillin-Resistant Staphylococci. 1988;1(2):173–86.

17. Hackbarth CJ, Chambers HF. Methicillin-Resistant Staphylococci : Detection Methods and Treatment of Infections. 1989;(July).

18. Tanaka T, Okuzumi K, Iwamoto A, Hiramatsu K. A retrospective study of methicillin-resistant Staphylococcus aureus clinical strains in Tokyo University Hospital. J Infect Chemother. 1995;1(1):40–9.

19. Caierão J, Musskopf M, Superti S, Roesch E, Dias CG, D'Azevedo PA. Evaluation of phenotypic methods for methicillin resistance characterization in coagulase-negative staphylococci (CNS). J Med Microbiol. 2004;53(12):1195–9.

20. Jain A, Agarwal A, Verma RK. Cefoxitin disc diffusion test for detection of meticillin-resistant staphylococci. J Med Microbiol. 2008;57(8):957–61.

21. Bignardi GE, Woodford N, Chapman A, Johnson AP, Speller DCE. Detection of the mec-A gene and phenotypic detection of resistance in Staphylococcus aureus isolates with borderline or low-level methicillin resistance. 1996;53–63.

22. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Meticillin-resistant Staphylococcus aureus (MRSA): global epidemiology and harmonization of typing methods. Int J Antimicrob Agents. 2012 Apr 1;39(4):273–82.

23. Omuse G, Kabera B. Low prevalence of methicillin-resistant as determined by an automated identification system in two private hospitals in Nairobi, Kenya : a cross-sectional study Low prevalence of methicillin-resistant Staphylococcus aureus as determined by an automated i. 2014;14(January):1–6.

24. Maina EK, Kiiyukia C, Wamae CN, Waiyaki PG, Kariuki S. International Journal of Infectious Diseases Characterization of methicillin-resistant Staphylococcus aureus from skin and soft tissue infections in patients in Nairobi, Kenya. Int J Infect Dis. 2013;17(2):e115–9.

25. Fernandes CJ, Fernandes LA, Collignon P, Bradbury S, Gottlieb T, Funnell G, et al. Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother. 2005 Apr;55(4):506–10.

26. Haddadin AS, Fappiano SA, Lipsett PA. Staphylococcus aureus. 2002;385–92.

27. Ecdc. SURVEILLANCE REPORT: Antimicrobial resistance surveillance in Europe 2014. 2014;

28. Hassoun A, Linden PK, Friedman B. Incidence, prevalence, and management of MRSA bacteremia across patient populations review of recent developments in MRSA management and treatment. Crit Care Lond Engl. 2017 Aug 14;21(1):211.

29. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. Lancet 368: 874-885. Lancet. 2006 Oct 1;368:874–85.

30. Stefani S, Varaldo PE. Epidemiology of methicillin-resistant staphylococci in Europe. Clin Microbiol Infect. 2003;9(12):1179–86.

31. Report G. Antimicrobial resistance. 2014;

32. Kateete DP, Namazzi S, Okee M, Okeng A, Baluku H, Musisi NL, et al. High prevalence of methicillin-resistant Staphylococcus aureus in the surgical units of Mulago Hospital in Kampala, Uganda. BMC Res Notes. 2011;4(1):326.

33. Falagas ME, Karageorgopoulos DE, Leptidis J, Korbila IP. MRSA in Africa : Filling the Global Map of Antimicrobial Resistance. 2013;8(7).

34. Access O. Research article A study on antimicrobial susceptibility pattern in clinical isolates of Staphylococcus aureus in Eritrea. 2009;8688:1–5.

35. Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant Staphylococcus aureus. Trends Microbiol. 2001;9(10):486–93.

36. Galia L, Ligozzi M, Bertoncelli A, Mazzariol A. Real-time PCR assay for detection of Staphylococcus aureus, Panton-Valentine Leucocidin, and Methicillin Resistance directly from clinical samples. 2019;5(May):138–46.

37. Utsui Y, Yokota T. Role of an Altered Penicillin-Binding Protein in Methicillin-and Cephem-Resistant Staphylococcus aureus. Vol. 28, ANTIMICROBIAL AGENTS AND CHEMOTHERAPY. 1985 p. 397–403.

38. Berger-Bächi B, Tschierske M. Role of fem factors in methicillin resistance. Drug Resist Updat. 1998 Jan 1;1(5):325–35.

39. Nikaido H. Multidrug resistance in bacteria. Annu Rev Biochem. 2009;78:119–46.

40. Stapleton PD, Taylor PW. Methicillin resistance in Staphylococcus aureus: mechanisms and modulation. Vol. 85, Science Progress. 2002 p. 57–72.

41. Hiramatsu K, Katayama Y, Yuzawa H, Ito T. Molecular genetics of methicillin-resistant Staphylococcus aureus [Internet]. Vol. 292, Int. J. Med. Microbiol. 2002. Available from: http://www.urbanfischer.de/journals/ijmm

42. Hiramatsu K, Katayama Y, Matsuo M, Sasaki T, Morimoto Y, Sekiguchi A, et al. Multi-drug-resistant Staphylococcus aureus and future chemotherapy. J Infect Chemother. 2014 Oct 1;20(10):593–601.

43. David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: Epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev. 2010;23(3):616–87.

44. RodrÃ\-guez-Noriega E, Seas C. The changing pattern of methicillin-resistant staphylococcus aureus clones in Latin America: implications for clinical practice in the region. Braz J Infect Dis. 2010;14:87–96.

45. Janout V. CURRENT KNOWLEDGE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AND COMMUNITY-ASSOCIATED

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS Ivanka Matouskova, Vladimir Janout. 2008;152(2):191–202.

46. Meyer F, Girardot R, Pie Y, Pre G, Colin DA. Analysis of the Specificity of Panton-Valentine Leucocidin and Gamma-Hemolysin F Component Binding . 2009;77(1):266–73.

47. Vandenesch F, Lina G, Gillet Y, Etienne J, Crémieux AC. [The end of the controversy: Panton-Valentine is the culprit]. Med Sci MS. 2009 Nov;25(11):984—986.

48. Mcdonald RR, Antonishyn NA, Hansen T, Snook LA, Nagle E, Mulvey MR, et al. Development of a Triplex Real-Time PCR Assay for Detection of Panton-Valentine Leukocidin Toxin Genes in Clinical Isolates of Methicillin-Resistant Staphylococcus aureus. 2005;43(12):6147–9.

49. Rutare S. Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) among pediatric patients admitted in intensive care unit and neonatal intensive care unit at Kenyatta National hospital-Nairobi, Kenya. 2013;(August).

50. Wangai FK, Masika MM, Maritim MC, Seaton RA. Methicillin-resistant Staphylococcus aureus (MRSA) in East Africa : red alert or red herring ? 2019;1–10.

51. Gitau W, Masika M, Musyoki M, Museve B, Mutwiri T. Antimicrobial susceptibility pattern of Staphylococcus aureus isolates from clinical specimens at Kenyatta National Hospital. BMC Res Notes. 2018;11(1):1–5.

52. Hussain Z, Stoakes L, Garrow S, Longo S, Fitzgerald V, Lannigan R. Rapid Detection of mecA -Positive and mecA -Negative Coagulase-Negative Staphylococci by an Anti-Penicillin Binding Protein 2a Slide Latex Agglutination Test. 2000;38(6):2051–4.

53. Diederen B, Duijn IV, Belkum AV, Willemse P, Keulen PV, Kluytmans J. Performance of CHROMagar MRSA Medium for Detection of Methicillin-Resistant Staphylococcus aureus. 2005;43(4):1925–7.

54. Sperber WH, Tatini SR. Interpretation of the Tube Coagulase Test for Identification of Staphylococcus aureus. Appl Microbiol. 1975;29(4):502–5.

55. Datta P, Gulati N, Singla N, Vasdeva HR, Bala K. Evaluation of various methods for the detection of meticillin-resistant Staphylococcus aureus strains and susceptibility patterns Printed in Great Britain. 2011;1613–6.

56. CLSI. M100 Performance Standards for Antimicrobial. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

57. Velasco D, Tomas M, Cartelle M, Beceiro A, Perez A, Molina F, et al. Evaluation of different methods for detecting methicillin. 2005;55(3):379–82.

58. Becker K, Pagnier I, Schuhen B, Wenzelburger F, Friedrich AW, Kipp F, et al. Does nasal cocolonization by methicillin-resistant coagulase-negative staphylococci and methicillin-susceptible Staphylococcus aureus strains occur frequently enough to represent a risk of false-positive methicillin-resistant S. aureus determinations by molecular methods? J Clin Microbiol. 2006 Jan;44(1):229–31.

59. Boyce JM, White RL, Bonner MC, Lockwood WR. Reliability of the MS-2 System in Detecting Methicillin-Resistant Staphylococcus aureus. 1982;15(2):220–5.

60. Tiwari HK, Sapkota D, Das AK, Sen MR. Assessment of different tests to detect methicillin-resistant Staphylococcus aureus. Southeast Asian J Trop Med Public Health. 2009;40(4):801–6.

61. Barry AL, Badal RE. Reliability of the Microdilution Technic for Detection of Methicillin-resistant Strains of Staphylococcus aureus. Am J Clin Pathol. 1977 May 1;67(5):489–95.

62. Cleary TJ, Maurer D. Methicillin-Resistant Staphylococcus aureus Susceptibility Testing by an Automated System, Autobac I. 1978;13(5):837–41.

63. Drew WL, Barry AL, Toole RO, Sherris JC. Reliability of the Kirby-Bauer Disc Diffusion Method for Detecting Methicillin-Resistant Strains of Staphylococcus aureus. 1972;24(2):240–7.

64. Thornsberry C, Caruthers JQ, Baker CN. Effect of Temperature on the In Vitro Susceptibility of Staphylococcus aureus to Penicillinase-Resistant Penicillins. 1973;4(3):263–9.

65. Coudron PE, Jones DL, Dalton HP, Archer GL. Evaluation of Laboratory Tests for Detection of Methicillin-Resistant Staphylococcus aureus and Staphylococcus epidermidis. 1986;24(5):764–9.

66. Hansen SL, Freedy PK, Lilly E. Variation in the Abilities of Automated , Commercial , and Reference Methods to Detect Methicillin-Resistant (Heteroresistant) Staphylococcus aureus. 1984;20(3):494–9.

67. Ligozzi M, Bernini C, Bonora MG, Fatima MD, Zuliani J, Fontana R. Evaluation of the VITEK 2 System for Identification and Antimicrobial Susceptibility Testing of Medically Relevant Gram-Positive Cocci. 2002;40(5):1681–6.

68. Francisco S. Performance of Vitek 2 for Antimicrobial Susceptibility Testing of. 2014;52(2):392–7.

69. Nonhoff C, Roisin S, Hallin M, Denis O. Evaluation of Clearview exact PBP2a, a new immunochromatographic assay, for detection of low-level methicillin-resistant Staphylococcus aureus (LL-MRSA). J Clin Microbiol. 2012;50(10):3359–60.

70. Otte KM, Jenner S, Wulffen HV. Identification of methicillin-resistant Staphylococcus aureus (MRSA): Comparison of a new molecular genetic test kit (GenoType® MRSA) with standard diagnostic methods. Clin Lab. 2005;

71. Kobayashi N, wu H, Kojima K, Taniguchi K, Urasawa S, Uehara N, et al. Detection of mecA, femA, and femB genes in clinical strains of staphylococci using polymerase chain reaction. Epidemiol Infect. 1994;113(2):259–66.

72. Mrsa GT. GenoType MRSA. 2017;Instructio:1–10.

73. Hussain M, Grundmeier M, Bru M, Lo B, Varga G, Roth J, et al. Staphylococcus aureus Panton-Valentine Leukocidin Is a Very Potent Cytotoxic Factor for Human Neutrophils. 2010;6(1).

74. DuMont AL, Yoong P, Surewaard BGJ, Benson MA, Nijland R, Strijp JAG van, et al. Staphylococcus aureus elaborates leukocidin AB to mediate escape from within human neutrophils. Infect Immun. 2013;81(5):1830–41.

75. Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant Staphylococcus aureus: The role of Panton-Valentine leukocidin. Lab Invest. 2007;87(1):3–9.

76. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. Staphylococcus aureus isolates carrying panton-valentine leucocidin genes in England and Wales: Frequency, characterization, and association with clinical disease. J Clin Microbiol. 2005;43(5):2384–90.

77. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant staphylococcus aureus carrying panton-valentine leukocidin genes: Worldwide emergence. Emerg Infect Dis. 2003;9(8):978–84.

78. Breurec S, Fall C, Pouillot R, Boisier P, Brisse S, Diene-Sarr F, et al. Epidemiology of methicillin-susceptible Staphylococcus aureus lineages in five major African towns: High prevalence of Panton-Valentine leukocidin genes. Clin Microbiol Infect. 2011;17(4):633–9.

79. Köck R, Mellmann A, Schaumburg F, Friedrich AW, Kipp F, Becker K. The Epidemiology of Methicillin-Resistant Staphylococcus aureus (MRSA) in Germany. Dtsch Aerzteblatt Online. 2011;108(45).

80. Shallcross LJ, Williams K, Hopkins S, Aldridge RW, Johnson AM, Hayward AC. Panton–Valentine leukocidin associated staphylococcal disease : a cross-sectional study at a London hospital, England. Eur Soc Clin Infect Dis. 2010;16(11):1644–8.

81. Omuse G, Shivachi P, Kariuki S, Revathi G. Prevalence of Panton Valentine Leukocidin in Carriage and Infective Strains of <i>Staphylococcus aureus</i> at a Referral Hospital in Kenya. Open J Med Microbiol. 2013;03(01):5–11.

82. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, et al. Survey of Infections Due to Staphylococcus Species : Frequency of Occurrence and Antimicrobial Susceptibility of Isolates Collected in the United States, Canada, Latin America, Europe, and the Western Pacific Region for the SENTRY Antimicrobial Survei. 2001;52242(Suppl 2).

83. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America for the Treatment of Methicillin-Resistant Staphylococcus aureus Infections in Adults and Children. 2011;52.

84. Khan A, Wilson B, Gould IM. Current and future treatment options for community-associated MRSA infection. Expert Opin Pharmacother. 2018 Mar 24;19(5):457–70.

85. Mahjabeen F, Saha U, Mostafa MN, Siddique F, Ahsan E, Fathma S, et al. An Update on Treatment Options for Methicillin-Resistant Staphylococcus aureus (MRSA) Bacteremia: A Systematic Review. Cureus. 2022 Nov 14;

86. Pournajaf A, Ardebili A, Goudarzi L, Khodabandeh M, Narimani T, Abbaszadeh H. PCR-based identification of methicillin-resistant Staphylococcus aureus strains and their antibiotic resistance profiles. Asian Pac J Trop Biomed. 2014;4(1067):S293–7.

87. Quan KA, Sater MRA, Uy C, Clifton-Koeppel R, Dickey LL, Wilson W, et al. Epidemiology and genomics of a slow outbreak of methicillin-resistant Staphylococcus aureus (MRSA) in a neonatal intensive care unit: Successful chronic decolonization of MRSA-positive healthcare personnel. Infect Control Hosp Epidemiol. 2023 Apr 16;44(4):589–96.

88. Popovich KJ, Davila S, Chopra V, Patel PK. Annals of Internal Medicine S SUPPLEMENT : STRIVE A Tiered Approach for Preventing Methicillin-Resistant Staphylococcus aureus Infection. 2019;

89. Vernon MO, Hayden MK, Trick WE, Hayes RA. Chlorhexidine Gluconate to Cleanse Patients in a Medical Intensive Care Unit. 2006;166.

90. Committee A, Apic S. Morbidity and Mortality Weekly Report Guideline for Hand Hygiene in Health-Care Settings Recommendations of the Healthcare Infection Control Practices Centers for Disease Control and Prevention TM. 2002;51.

91. Lemeshow S, Jr DWH, Klar J, Lwanga SK. Stanley Lemeshow, David W Hosmer Jr, Janelle Klar, and Stephen K. Lwanga.

92. Jauset-Rubio M, Svobodová M, Mairal T, McNeil C, Keegan N, Saeed A, et al. Ultrasensitive, rapid, and inexpensive detection of DNA using paper-based lateral flow assay. Sci Rep. 2016 Nov 25;6.

93. Kejela T, Dekosa F. High prevalence of MRSA and VRSA among inpatients of Mettu Karl Referral Hospital, Southwest Ethiopia. Trop Med Int Health. 2022 Aug 1;27(8):735–41.

94. Abubakar U, Sulaiman SAS. Prevalence, trend and antimicrobial susceptibility of Methicillin-Resistant Staphylococcus aureus in Nigeria: a systematic review. J Infect Public Health. 2018 Nov 1;11(6):763–70.

95. Sakoulas G, Gold HS, Venkataraman L, Degirolami PC, Eliopoulos GM, Qian Q. Methicillin-resistant Staphylococcus aureus: Comparison of susceptibility testing methods and analysis of mecA-positive susceptible strains. J Clin Microbiol. 2001;39(11):3946–51.

96. Girgis SA, Gomaa HE, Saad NE, Salem MM. A comparative study for detection of methicillin resistance staphylococci by polymerase chain reaction and phenotypic methods. Life Sci J. 2013;10(4):3711–8.

97. Felten A, Grandry B, Lagrange PH, Casin I. Evaluation of three techniques for detection of low-level methicillin-resistant Staphylococcus aureus (MRSA): A disk diffusion

method with cefoxitin and moxalactam, the vitek 2 system, and the MRSA-screen latex agglutination test. J Clin Microbiol. 2002;40(8):2766–71.

98. Hirvonen JJ. The use of molecular methods for the detection and identification of methicillin-resistant Staphylococcus aureus. Biomark Med. 2014 Oct 1;8(9):1115–25.

99. Witte W, Pasemann B, Cuny C. Detection of low-level oxacillin resistance in mecA-positive Staphylococcus aureus. Clin Microbiol Infect. 2007;13(4):408–12.

100. Ellington MJ, Hope R, Ganner M, Ganner M, East C, Brick G, et al. Is Panton–Valentine leucocidin associated with the pathogenesis of Staphylococcus aureus bacteremia in the UK ? 2007;(June):402–5.

101. Bhatta DR, Cavaco LM, Nath G, Kumar K, Gaur A, Gokhale S, et al. Association of Panton-Valentine Leukocidin (PVL) genes with methicillin-resistant Staphylococcus aureus (MRSA) in Western Nepal: A matter of concern for community infections (a hospital-based prospective study). BMC Infect Dis. 2016;16(1):1–6.

102. Lo B, Niemann S, Ehrhardt C, Horn D, Lanckohr C, Lina G, et al. Pathogenesis of Staphylococcus aureus necrotizing pneumonia : the role of PVL and an influenza coinfection. 2013;1041–51.

APPENDICES

Appendix I: DATA COLLECTION TOOLS

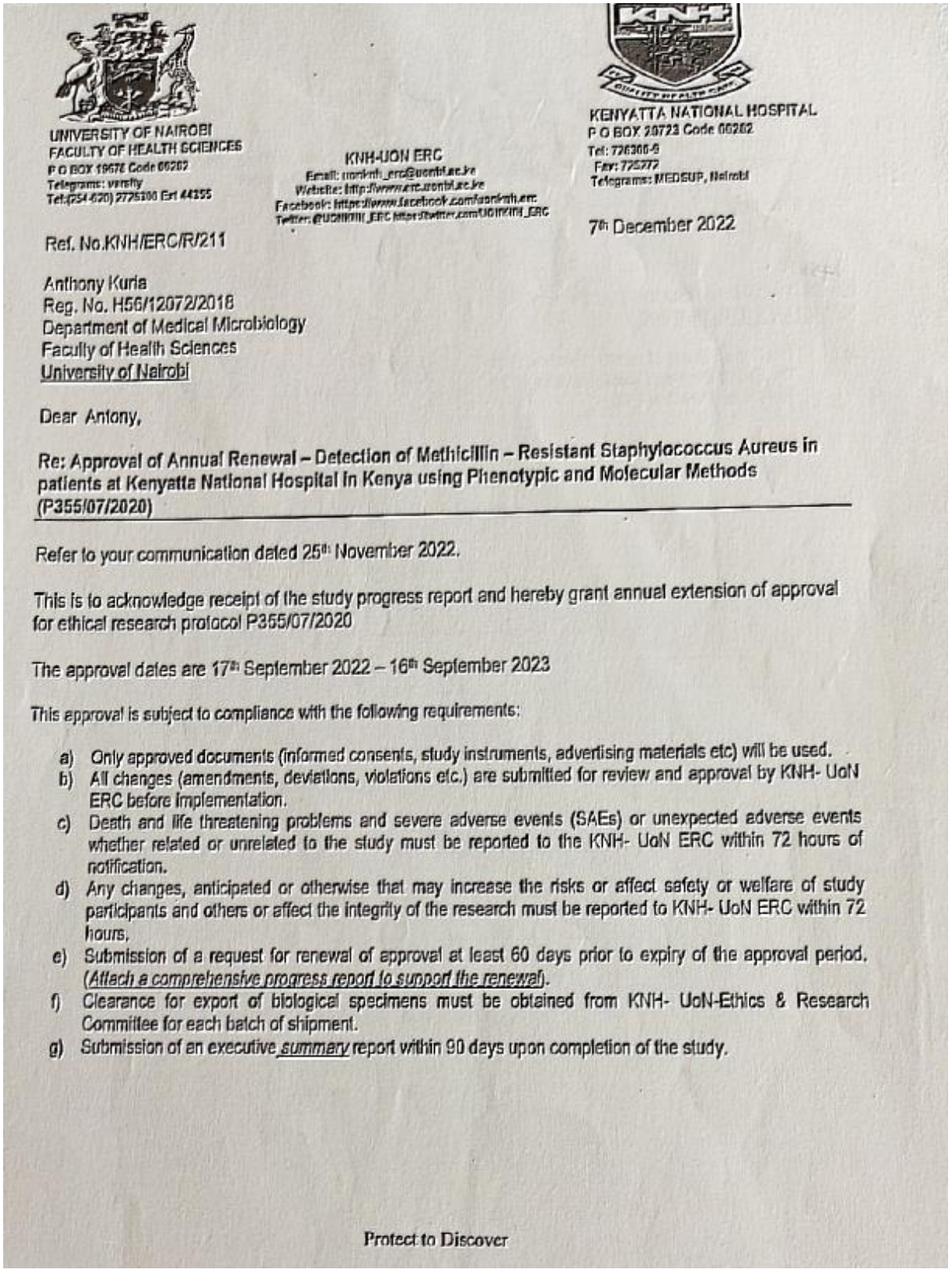
Isolate data collection tool

KNH –MICROBIOLOGY DATA COLLECTION FORM 1 OXACILLIN RESISTANT (MIC > 4µg /CEFOXITIN POSITIVE) STAPHYLOCOCCUS AUREUS									
SERIAL NO.	SPECIMEN TYPE	IP NO	AGE	SEX	WD / CLINIC	DATE	GROWTH ON		GENO-TYPE PCR RESULT
							CHROM agar	SBA	

Vitek 2 data collection tool

Selected organism: Staphylococcus aureus		
SUSCEPTIBILITY INFORMATION		
ANTIMICROBIAL	MIC	INTERPRETATION
Cefoxitin screen		
Benzylpenicillin		
Oxacillin		
Gentamycin		
Levofloxacin		
Erythromycin		
Clindamycin		
Linezolid		
Teicoplanin		
Vancomycin		
Tetracycline		
Trimethoprim/Sulfamethoxazole		

Appendix II: UON-KNH ERC APPROVAL LETTER



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Ref. No.KNH/ERC/R/211

7th December 2022

Anthony Kuria
Reg. No. H56/12072/2018
Department of Medical Microbiology
Faculty of Health Sciences
University of Nairobi

Dear Antony,

Re: Approval of Annual Renewal – Detection of Methicillin – Resistant Staphylococcus Aureus in patients at Kenyatta National Hospital in Kenya using Phenotypic and Molecular Methods (P355/07/2020)

Refer to your communication dated 25th November 2022.

This is to acknowledge receipt of the study progress report and hereby grant annual extension of approval for ethical research protocol P355/07/2020

The approval dates are 17th September 2022 – 16th September 2023

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH- UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH- UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an executive *summary* report within 90 days upon completion of the study.

Protect to Discover

Appendix III: SAMPLE OF THE GENOTYPE MRSA PCR RESULT

Sample of the GenoType MRSA result