Phenotypic analysis and antibiotic susceptibility of methicillin-resistant
*Staphylococcus aureus* in Kiambu County, Kenya

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Abstract

Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA) causes illness to people and can be picked up from both healthcare facilities and the environment leading to high morbidity and mortality. The study was aimed at identifying phenotypic characteristics of Methicillin-Resistant *Staphylococcus aureus* and determine the antibiotic susceptibility pattern of clinical samples isolated from patients attending or admitted in two health facilities in Kiambu County, Kenya.

Methodology: One hundred and thirty-eight (138) clinical samples were collected from patients attending Thika and Kiambu Level-5 Hospitals. The isolates were obtained using standard bacteriological techniques. Methicillin resistance of *Staphylococcus aureus* was determined using the cefoxitin disk diffusion test.

Results: 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.

Conclusions: The data obtained revealed that there is presence of MRSA in healthcare settings in Kiambu County, Kenya with varying antibiotic sensitivity patterns as well as multidrug resistance. The findings will help healthcare workers in the county to develop preventive strategy as well as institute policy for antibiotic usage, infection control and surveillance.

Key words: *Staphylococcus aureus*; phenotypic characterization; antibiotic susceptibility, methicillin resistance.


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Introduction

*Staphylococcus aureus* is a significant agent of disease causative agent in hospitals as well as community settings in the world [1]. Prior to the advent of antimicrobial agents, *S. aureus* infection was fatal until the discovery of penicillin that brought great relief to the patients. However, soon after the roll-out of these drugs, resistance appeared due to β-lactamases production [2]. Subsequently, methicillin was introduced to tackle β-lactamase degradation (β-lactam resistance). Unfortunately, the bacteria became resistant to methicillin too and started being termed methicillin-resistant *Staphylococcus aureus* (MRSA) [2,3]. This results in increased morbidity and mortality in hospitals and also in selection of MRSA [2-3]. Resistance to methicillin implies resistance to antimicrobial agents such as penicillins and cephalosporins, which are referred to as β-lactams, with unique chromosomal integration locus encoding penicillin-binding protein (PBP) [4-5]. A variety of conditions including septicemia, wound sepsis and pneumonia are caused by MRSA, posing a grave threat to public health both in the community and hospitals because of resistance to anti-staphylococcal antibiotics [4].

The prevalence rates of MRSA have been reported to be over 50% in most regions of the world such as Asia, Malta, North and South America as well as Europe that has average prevalence of 25% with higher prevalence of 54% reported in Portugal, 43 - 58% in Italy and the lowest prevalence of 2% in Switzerland [6-7]. In Africa, most countries, including Ethiopia, Kenya, Nigeria, Senegal, South Africa and South Sudan, reported the emergence of MRSA in the mid 80’s [8]. MRSA incidence in most African countries was found to be about 15% with higher incidences of
up to 30% being reported in Nigeria, Cameroon and Kenya [9]. However, MRSA incidence rates have been reported to be below 10% in Algeria and Tunisia [10].

The epidemiology of MRSA especially Community-acquired Methicillin-Resistant Staphylococcus aureus (CA-MRSA) clones is more complex due to their spread and dissemination in hospitals [11]. The increasing prevalence of MRSA infections due to the emergence of unique community-associated S. aureus strain is a global problem [11]. In developing countries, such as Kenya, little is known about the characteristics and frequency of MRSA compared to developed countries [11]. MRSA has a type of penicillin-binding protein (PBP2a) that makes it resistant to all available \( \beta \)-lactam antibiotics. Colonized patients are important reservoirs of MRSA in hospitals/communities. However, the current diagnostic tools can miss between 35%–84% of these colonized patients [12]. The level of endemicity of MRSA determines the kind of screening program needed in order not to miss occult carriers of MRSA which could lead to increased cross-transmission of MRSA in hospital infections. This study was aimed at characterization of MRSA phenotypes- as hospital- or community-acquired, level of MRSA colonization and antimicrobial susceptibility patterns in Kiambu County. Kiambu County was chosen for this study because of the cosmopolitan nature of its population which represents all regions of the country as well as the high incidence of motorcycle accidents resulting in wounds which are a good environment for MRSA colonization. Motorcycles have recently become a very common cheap way of transport in Kenya. However, this has brought with it a serious health challenge due to the many number of motorcycle accidents. Motorcyclists are known to operate without the right personal gear which means that, when they get into accidents- and it is often, they suffer wounds and bone fractures some of them life-threatening. These wounds provide a fertile ground for bacterial infections including MRSA. The study will provide clinicians, other healthcare professionals as well as policy-makers with relevant information on how to control, manage and reduce the spread of infections. This is important to both the people already with MRSA colonies and those, for instance, with wounds not yet colonized by MRSA and other vulnerable populations.

Methodology

Study Design

This is a cross-sectional descriptive laboratory-based study involving inpatients and outpatients in two health facilities in Kenya, namely, Thika and Kiambu Teaching and Referral hospitals between conducted between December 2017 and September 2018.

The Study Area

Kiambu County is located within greater Nairobi, adjacent to the northern border of Nairobi County. Its capital is Kiambu and its largest town is Thika. It has a total area of 2,543.4 square km with a total population of 1,623,282 in 496,244 households, with a population density of 638 per square km. Thika Teaching and Referral Hospital is a 300-bed Government Hospital in the town of Thika while Kiambu Teaching and Referral Hospital is a 289-bed Government Hospital located in Kiambu East District, Kiambu County [4]. The two hospitals were chosen due to their large catchment areas from the whole County and also due to the fact that they handle both outpatients and inpatients.

Study Population and sample size

The population of Kiambu County is mixed urban and rural. The patients in this study residents in the County, admitted or seeking services in the 2 health facilities. The study’s sample size was calculated using MRSA prevalence of 8.9% obtained in a previous study by [4] in Thika, Kenya. This gave a sample size of 138 samples at 95% confidence level.

Inclusion Criteria

Inclusion criteria were: presentation with wound or purulent discharge during inpatient or outpatient services; children and adults irrespective of age; ability to understand risks and benefits of the study; consent and agreement by patient or guardians (in case of children); signing or thumb-printing informed consent form; and patients residing in the County and seeking services in the 2 health facilities.

Exclusion Criteria

Patients (or their relative) who declined to sign the informed consent form or patients not residing in the County and not admitted to the two health facilities were excluded.

Ethical considerations and approvals

Ethical clearance was given by Mount Kenya University Ethical Review Committee. Clearance was also sought and received from the National Commission for Science Technology and Innovation, County Commissioner, County Director of Health services as well as the Medical Superintendents of Thika-Teaching and Referral Hospital and Kiambu
Teaching and Referral Hospital. Subjects or their guardians who participated in the study were made to understand the risks and benefits of the study and thereafter asked to sign or thumbprint an informed consent form prior to sample collection. All samples collected were treated with utmost confidentiality.

**Sample Collection**

Sterile dry sterile cotton swabs (Himedia Laboratories, Mumbai, India) were used in the collection of samples from infected sites such as cellulitis, skin abscesses, infected skin ulcers, infected surgical ulcers, ischemic ulcers, osteomyelitis, and burns. After collection of samples the swabs were placed in sheaths and transported to the laboratory for inoculation.

**Culture of Samples**

The samples were inoculated into 5% sheep blood agar plates obtained commercially from Kenya Medical Research Institute (KEMRI), Nairobi and incubated at 37°C for 24 hours. *Staphylococcus aureus* colonies from sheep blood agar were inoculated into mannitol salt agar plates (KEMRI, Nairobi) and incubated at 37 °C for 24 hours before checking for fermentation of mannitol. All positive isolates from mannitol agar were sub-cultured into Mueller-Hinton Agar (Himedia Laboratories, Mumbai India) and incubated at 37 oC for 24 hours to obtain pure colonies. The isolates were confirmed by colony morphology, Gram stain and biochemical assays for catalase and coagulase using rabbit plasma. (Himedia, Mumbai India). Pure cultures of *S. aureus* obtained were kept at -20 °C freezer. Amounts of 1 ml of Tryptic Soy Broth (TSB) (KEMRI, Nairobi) were placed into 3 cryovials (Sarstedt, Numbrecht, Germany) per isolate and colonies of *S. aureus* were applied into the tubes and mixed. The *S. aureus* suspensions were stored at -80 °C freezer until needed for further analysis.

**Detection of Methicillin Resistance and Antibiotic Susceptibility Testing**

**Detection of Methicillin Resistance for suspected MRSA by Cefoxitin-Based Method**

Detection of methicillin resistance by *S. aureus* was carried out by disk-diffusion (Kirby-Bauer) technique [13,14] with 30 µg cefoxitin (Liofilchem, Roseto degli Abruzzi, Italy) according to the Clinical Laboratory Standard Institute (CLSI) guidelines [15].

**Antibiotic Susceptibility Testing for MRSA**

The antimicrobial susceptibility testing of *S. aureus* isolates to antibiotics was carried out by disk diffusion (Kirby-Bauer) [13,14] according to the CLSI guidelines [15]. Multi discs with nine (9, including cefoxitin) antimicrobial agents (Himedia Laboratories, Mumbai India) were used, namely: penicillin G – 1 i.u.; minocycline – 30 mcg; erythromycin – 15 mcg; methicillin – 5 mcg; co-Trimoxazole – 25 mcg; chloramphenicol – 30 mcg; ampicillin – 10 mcg; lincomycin 2 mcg. The *S. aureus* ATCC 25923 clone was used as a positive control.

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<th>Table 1. Prevalence of <em>Staphylococcus aureus</em> by gender, age groups, hospitals and hospital units.</th>
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Values are expressed in percentages and frequency (Table 1 values in brackets). Pearson Chi Square or Fisher’s exact test was used to compute for associations between different variables followed by Phi or Cramer’s V to measure the association. The p values ≤ 0.05 were considered significant. OPD = Outpatient department, N = Total number of samples.
Colony Saline Suspension Preparation of S. aureus

Two milliliters of 0.85% normal saline was placed into sterile tubes and 2 – 3 colonies of S. aureus were added and mixed. The suspensions obtained were checked visually for turbidity and compared with 0.5 McFarland Standard.

Plate Inoculation, Disk dispensing and Reading

Swabs were briefly soaked in the bacterial suspensions and streaked onto MHA plates after removing excess solution. Antibiotic disks were dispensed into streaked MHA and read after incubation for 18 – 24 hours. Plates were read using a ruler and reported in millimeters according to CLSI guidelines.

Results

Prevalence of Staphylococcus aureus in samples

A total of 138 samples were collected (78 males and 60 females aged 1 – 80 years) in both Kiambu (91/138) and Thika Level-5 Hospitals (47/138). Of the 138 samples collected in the two hospitals in Kiambu County, Kenya, 54 samples were Staphylococcus aureus culture positive giving a prevalence of 39.1% (95% CI = 30.1 - 46.4; \( P < 0.00001 \)) as shown in Table 1. Antimicrobial sensitivity of all the 54 S. aureus-positive isolates was tested on 9 common antibiotics. Of the 9 antibiotics tested, 7 (77.8%) were observed to have resistance levels of between 30-85% against the isolates.

Prevalence of MRSA in samples

MRSA was detected in 22 (40.7%) out of 54 samples based on cefoxitin-based susceptibility test. The overall prevalence of MRSA from the total of 138 samples collected was 15.9% (22/138; 95% CI = 9.4 - 22.1; \( P < 0.00001 \)) as presented in Table 2.

Prevalence of MRSA based on Cefoxitin susceptibility by gender

There was no significant association between MRSA prevalence and the gender of patients based on cefoxitin susceptibility in Kiambu County, Kenya (Table 2; \( \chi^2 = 2.825; \text{df} = 1; P = 0.09 \)). The relationship

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Values are expressed in percentages and frequency (values in brackets). Pearson Chi Square or Fisher’s exact test was used to compute for associations between different variables followed by Phi or Cramer’s V to measure the association. The p values ≤0.05 were considered significant. OPD = Outpatient department, N = Total number of samples; MRSA = Methicillin resistant Staphylococcus aureus.
between gender and MRSA prevalence had a weak association (Table 2; Phi = -0.14). The female patients had a higher prevalence of 22.0% (13/59), while male patients had a prevalence of 11.4% (9/79) (Figure 1; Table 2). The prevalence of MRSA in female patients was 2.20 (Odd ratio; 95% CI = 0.87-5.56; P = 0.09) times greater compared to male patients based on the Cefoxitin susceptibility test.

Prevalence of MRSA based on Cefoxitin susceptibility by age groups in years

The association between age groups of the patients and MRSA prevalence was not statistically significant in Kiambu County, Kenya (Table 2; Fisher’s exact test; P = 0.38). The relationship between the age groups of patients exhibited a weak association (Table 6; Cramer’s V = 0.15). The age groups between 1-20, 21-40, 41-60 and 61-80 years old had a MRSA prevalence of 18.9% (7/37), 12.7% (8/63), 12.0% (3/25) and 30.8% (4/13) respectively (Figure 2; Table 2).

Prevalence of MRSA based on Cefoxitin susceptibility by hospitals

There was no significant association between the hospitals and the prevalence of MRSA in Kiambu County (Table 2; χ² = 1.496; df = 1; P = 0.22). The relationship between the hospitals and MRSA prevalence exhibited a weak association (Table 2; Phi = 0.10). The Kiambu hospital recorded a MRSA prevalence of 18.7% (17/91), while Thika hospital had a prevalence of 10.6% (5/47) (Figure 3; Table 2). The MRSA prevalence of the patients attending Kiambu hospital was 1.93 (odd ratio; 95% CI = 0.66-5.61) times likely to be higher compared to Thika hospital.

Figure 3. Prevalence of MRSA based on Cefoxitin susceptibility by hospitals.

Prevalence of MRSA based on Cefoxitin susceptibility by hospital units

The association between hospital departments and MRSA prevalence was statistically significant in Kiambu County Kenya (Table 2; Fisher’s exact test; P = 0.01). The relationship between the hospital departments and the MRSA prevalence was moderately strong (Cramer’s V = 0.26). The outpatient department had the highest MRSA prevalence of 29.3% (12/41), followed by pediatric unit with a prevalence of 20.0% (3/15), while surgical unit recorded the least MRSA prevalence of 8.5% (7/82) (Figure 4; Table 2).

Antimicrobial Susceptibility Pattern of Staphylococcus aureus Isolates

Antimicrobial susceptibility using disk diffusion technique was performed on all 54 isolates of S. aureus...
using 9 antibiotics (Figure 5). The isolates were classified as either sensitive, intermediate or resistant according to the CLSI zone diameter interpretive criteria. Zone diameters were read using a ruler. The 54 S. aureus isolates demonstrated the following susceptibility patterns to the antibiotics: cefoxitin 32 (59.3%), penicillin G 8 (14.8%), minocycline 34 (63.0%), erythromycin 26 (48.1%), methicillin 28 (51.9%), co-trimoxazole 15 (27.8%), chloramphenicol 46 (85.2%), ampicillin 18 (33.3%) and lincomycin 33 (61.1%). The intermediate susceptibility pattern to the isolates shown: minocycline 4 (7.4%), methicillin 1 (1.9%), co-trimoxazole 4 (7.4%), ampicillin 7 (13.0%) and lincomycin 4 (7.4%); presented with the following resistance pattern: cefoxitin 22 (40.7%), penicillin G 46 (85.2%), minocycline 12 (22.2%), erythromycin 24 (44.4%), methicillin 25 (46.3%), co-trimoxazole 35 (66.8%), chloramphenicol 8 (15.1%), ampicillin 29 (53.7%) and lincomycin 17 (31.5%). Seven (7) of the 9 antibiotics (77.8%) had resistance levels between 30-85% against the isolates. The highest sensitivity was observed with chloramphenicol, with 46 (85.2%) and the lowest with penicillin G, having 8 (14.8%) and 46 (85.2%) resistance, respectively. Only one (1.9%) isolate was found to be sensitive to all 9 antibiotics tested while one (1.9%) isolate was observed to be resistant to all.

Multidrug Resistance

MDR was reported based on resistance to more than 3 antibiotics by bacterial isolate [16]. Among the 54 isolates of S. aureus, 35 (64.8%) were observed to be MDR (Figure 6). All 22 MRSA strains were found to be MDR.

Discussion

Out of 138 samples collected, 54 (39.1%) were observed to have Staphylococcus aureus. This is higher than the study conducted by Aiken et al. [4] at Thika Teaching and Referral Hospital, Kenya with a prevalence of 8.9% and Mbogori et al. [17] in Nairobi County, Kenya with a prevalence of 20%. However, the result is comparable with a prevalence of 37.4% of S. aureus observed in studies conducted in Nigeria by Ghebremedhin et al. [18] but lower than one conducted in Tanzania by Mohammed et al. [19] with a prevalence of 64.4%. S. aureus was found in all age groups of our study. The results from this study showed the carriage of the bacteria was not significantly associated with gender. From the two study sites, patients attending Kiambu Level five hospital had a higher prevalence of 40.7% S. aureus carriage in comparison with Thika Level-5 hospital that recorded 36.2%. There was a moderately strong association between prevalence of S. aureus and departments of the two hospitals, with outpatient and pediatric units having prevalence rates of 53.7% and 53.3%, respectively, compared to the surgical unit with 29.3% (Table 1).

MRSA was detected in 22 (40.7%) samples from 54 S. aureus isolates by cefoxitin-based susceptibility test in this study. This finding is consistent with previous studies that reported a prevalence of 31.5% in MRSA isolates in Uganda [20,21]. The facility-based study on MRSA conducted by Gitau et al., [22] in Kenyatta National Hospital (KNH) reported a MRSA prevalence of 27.8%. In this study, the prevalence of MRSA in

Figure 5. Antimicrobial susceptibility pattern of Staphylococcus aureus isolates.

Figure 6. Proportion of Multidrug Resistant (MDR) strains among the Staphylococcus aureus isolates.
Kiambu Hospital (24.2%) is in agreement with findings at KNH but 2.7 times higher than the 10.6% prevalence observed in Thika Level-5 hospital. However, even this 10.6% prevalence in Thika, is still higher than the 6.9% MRSA prevalence reported by Aiken et al., [4] in the same facility. This could point to a worrying trend in the rise of MRSA prevalence in the Thika Teaching and Referral Hospital since the earlier study in 2014. The bigger worry, however, should be on the higher prevalence of MRSA observed in the Kiambu facility which is almost 3 times higher compared to Thika and this may be due to congestion in the wards and outpatient clinic.

In other studies, a lower prevalence of 3.8% - in S. aureus isolates was obtained by Omuse et al. [23] from 2 private hospitals in Nairobi, Kenya, 15% by Mbogori et al. [17], from selected schools in Nairobi County and 7% by Aiken et al. [4] in Thika, Kenya. There is a statistically significant association between the prevalence of MRSA and the hospital departments where the samples were collected for this study. Samples collected from patients in the surgical unit revealed the lowest MRSA prevalence at 9.8%, while samples from patients in the outpatient and pediatrics units had the highest MRSA prevalence of 34.3% and 33.3%, respectively. In comparison, a study carried out by Maina et al., (11) revealed MRSA prevalence of 25% in outpatients and 75% MRSA prevalence in inpatients. With high prevalence of MRSA in the outpatient unit in this study, the risk of spreading the infection in the community even before arrival at the hospital is very possible and could be a challenge to the infection control effort of the County and Country at large.

Antibiotic susceptibility testing of the 54 S. aureus isolates was done on 9 common antibiotic drugs using the disc diffusion (Kirby-Bauer) technique according to the CLSI guidelines. In this study, resistance to penicillin, co-trimoxazole and ampicillin was observed to be over 50%. This could be due to the common and rampant use of these antibiotics in the community without prescription by authorized healthcare personnel. The highest sensitivity was recorded with chloramphenicol with sensitivity of 85.2%. There was an observed statistically significant difference in antibacterial susceptibility patterns of S. aureus isolates against penicillin, minocycline, erythromycin, methicillin, co-trimoxazole, chloramphenicol, ampicillin and lincomycin (p < 0.05) (figure 1). These findings are in agreement with those of a study by Mbogori et al., [17] where they observed co-trimoxazole resistance of over 50%. However, the results of the current study differ with the sensitivity of 100% obtained for tetracycline, erythromycin and ampicillin by that study [17]. Gitau et al., [22], in a study conducted at Kenyatta National Hospital reported penicillin resistance of 92% which is in agreement with the observed 85.2% figure of this study for S. aureus. In the susceptibility profiles in this study, all (100%) MRSA were resistant to Penicillin G. This observation is in agreement with the results of a study conducted in China by Wu et al., [24] that reported resistance of MRSA to penicillin G and ampicillin at 100% in comparison with Gitau et al. [22] with resistance of 97%.

Several studies demonstrated the occurrence of multidrug resistant S. aureus with cross-resistance to many antibiotics such as vancomycin, linezolid, methicillin and tigecycline [25]. In the study carried out in South Africa by Shittu et al. [26], over 80% of MRSA were resistant to more than 3 types of antibiotics. Many infections ranging from superficial to deep root have been found to be resistant to β-lactam antibiotics, clindamycin and erythromycin [27]. In this study, we found 35 of the 54 isolates (64.8%) of S. aureus in Kiambu County to be multidrug resistant (MDR). Resistance was highest in penicillin G (85.2%), co-trimoxazole (66.8%) and ampicillin (53.7%).

Conclusions

The data obtained in this study, revealed that there is presence of MRSA in healthcare settings in Kiambu County with varying antibiotic sensitivity patterns as well as multidrug resistance among the isolates. There is need for continuous research so as to monitor antibiotic susceptibility to S. aureus as well as MRSA to help regulate antibiotic resistance in the community. The findings will help healthcare workers in the community to develop preventive strategies against MRSA colonization as well as institute policy for antibiotic usage, infection control and surveillance so as to limit the spread of the infections in the county. In further study, we would look at the detection of specific resistant genes for mec A-mediated resistance in S. aureus so as to identify MRSA clones circulating in the County.

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isolation and antimicrobial susceptibility testing of the isolates.

Authors' Contributions
This work was carried out in collaboration between all authors. Authors SI, JM, RM and MM designed the study. Author SI recruited the study populations and performed the laboratory analysis. Authors SI, JM, RM and MM performed statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

Consent
Informed consent was obtained from the patients or guardians (in case of the children) to participate in the study by signing or thumb-printing informed consent form.

References


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**Conflict of interests:** No conflict of interests is declared.