

*Full Length Research Paper*

# **Antimicrobial susceptibility patterns of *Staphylococcus aureus* in a tertiary referral hospital in Nairobi, Kenya**

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***Staphylococcus aureus* is a leading cause of hospital and community acquired infections globally. Methicillin-resistant *S. aureus* (MRSA) prevalence has been reported to be high in various settings and is associated with increased morbidity, mortality and risk of nosocomial outbreaks. Surveillance of prevalence and antibiotic susceptibility patterns is important to ensure appropriate antibiotic prescription. The objective of our study was to establish the prevalence of *S. aureus*, to identify patterns of susceptibility to commonly used antibiotics and quantify contemporary penicillin resistance among *S. aureus*. A retrospective study was conducted at the Mater Hospital, Nairobi. The study involved a review of non-duplicate records of specimens analyzed between January 2014 and December 2018. An isolate was categorized as Penicillin susceptible (PSSA) if it was susceptible to Penicillin and Oxacillin, Methicillin susceptible (MSSA) if resistant to Penicillin but susceptible to Oxacillin and Methicillin resistant (MRSA) if resistant to Oxacillin and Penicillin. We present proportions of *S. aureus* that was PSSA, MSSA and MRSA. Multivariate logistic regression was used to determine the association between the presence of *S. aureus* isolates and the source of the clinical specimen (inpatient vs. outpatient), age and gender. A total of 659 isolates of *S. aureus* were analyzed in 5-year period. PSSA was the most prevalent organism seen (60.85%) while MRSA was the least prevalent (0.61%). Most *S. aureus* were isolated in pus from wound swabs-644 (73. 3%). A significant increase in susceptibility of *S. aureus* to Penicillin and Amoxicillin-clavulanic acid was-observed during the study period. This study demonstrated a high prevalence of Penicillin Sensitive *S. aureus* and a low prevalence of MRSA.**

**Key words:** *Staphylococcus aureus*, antimicrobial, penicillin.

## **INTRODUCTION**

*Staphylococcus aureus* is among the most common and devastating human bacterial pathogens, estimated to cause about 20-30% of bloodstream and surgical site infections globally, as well as up to half of bone and joint

infections (Diekema et al., 2019). Antibiotic resistance has emerged as a major problem in the treatment of infections caused by *S. aureus*. Methicillin resistant *S. aureus* (MRSA) was first reported in the early 1960s,

after the introduction of Methicillin following the acquisition of the *mecA* containing *Staphylococcal* cassette chromosome *mec*(SCC*mec*) (Kanjiyal et al., 2018). MRSA strains are not only resistant to nearly all beta-lactams, but many have developed resistance to multiple other classes of antimicrobials (Diekema et al., 2000). The epidemiology of MRSA infections has been marked by sequential “waves” of epidemic clones spreading across geographic regions, nations, and continents. As MRSA has become endemic, the use of Vancomycin for therapy of invasive MRSA infections has increased, along with concerns about development of Vancomycin resistance (Limbago et al., 2014). The prevalence of MRSA blood infections in the United States and Europe has dropped in recent years (Sutter et al., 2016). Similarly, MRSA infection rate in Asia is also declining (Abubakar and Sulaiman, 2018). In contrast, recent data suggests that the prevalence of MRSA in most African countries is rising, although the rate is estimated to be below 50% (Falagas et al., 2013). The changing trajectory of MRSA infection in developed countries has been attributed to implementation of control interventions (Kanjiyal et al., 2018). Recent data also suggests that Penicillin susceptibility may be in a period of renaissance (Cheng et al., 2016).

Data on MRSA prevalence in Africa has been variable and inconsistent due to lack of effective and systematic routine surveillance systems (Wangai et al., 2019). The objective of our study was to establish the prevalence of *S. aureus*, to identify patterns of susceptibility of *S. aureus* to commonly used antibiotics and quantify contemporary penicillin resistance among *S. aureus* in a tertiary center in Nairobi, Kenya. This information is essential in guiding the choice of antibiotic therapy and will serve as a baseline for measuring the impact of interventions.

## MATERIALS AND METHODS

This study was carried out at the microbiological laboratory at the Mater hospital, a tertiary referral and training hospital in Nairobi, Kenya. A total of 9000 culture specimens were analyzed in the laboratory between 2014 and 2018. We reviewed data on all the *S. aureus* isolated during this period.

### Data and sample collection

This was a retrospective study based on electronic laboratory records from the Mater hospital microbiological laboratory. The following demographic information was extracted from the hospital administrative database about each patient- inpatient vs. outpatient status at time of sample collection, age and gender, and antimicrobial susceptibility profiles of *S. aureus*. Data from 2014 to

2018 was retrieved from the hospital medical and laboratory records and for 2018 from VITEK® 2 (bioMérieux, France) (bioMérieux. Vitek 2, bioMérieux, 2018) antibiotic susceptibility system imported to a database.

### Isolation and identification

Samples collected within the hospital and its satellite clinics are routinely cultured on Blood agar (Oxoid, Basingstoke, Hampshire, England) and Mannitol salt agar (MSA) (Oxoid Limited, UK). The isolates are identified to be *S. aureus* using gram stain, catalase and coagulase tests. Mannitol-fermenting, gram-positive bacteria appearing as grape-like clusters and exhibiting catalase and coagulase positivity are identified as *S. aureus*. Tests are performed *in vitro*, and the growth response of an isolated organism to a particular drug or drugs are measured. These tests are performed under standardized conditions so that the results are reproducible. The raw data is either in the form of a zone size or minimum inhibitory concentration (MIC). All the antibiotics used are Oxoid, UK products

### Susceptibility testing

After incubation, the diameters of the complete growth inhibition zones around each disk are measured. Pre-specified breakpoints are used to interpret the zone sizes and classify them as susceptible(S), intermediate (I), or resistant(R). The most common method employed by the Mater hospital diagnostic laboratory between 2014 and 2018 was a simpler agar disk diffusion test (Kirby–Bauer method).

Clinical Laboratory Standards Institute (CLSI) M100-S19 breakpoints were used for interpretation. Isolates that were “intermediate” by CLSI breakpoints were grouped with resistant isolates for all analyses. Methicillin resistance during this period was determined using Oxacillin. This method used in the Mater hospital laboratory was similar to methods of MRSA identification used elsewhere (Udobi et al., 2013). Isolates from a solution adjusted to 0.5 McFarland standard were spot inoculated onto Mueller Hinton agar (MHA) (Oxoid, Basingstoke, Hampshire, England) supplemented with 6 micrograms/ml oxacillin and 4% sodium chloride. The plates were incubated at 35°C for 24 h. The isolates that survived showing more than one colony were considered Methicillin resistant.

### Ethical approval

Approval for this retrospective study was obtained from the Ethics committee of the Mater hospital. All patient information was de-identified prior to analysis.

### Statistical methods

The data collected was analyzed using Statistical Package for Social Sciences (SPSS INC, Chicago, IL, USA) (IBM-SPSS, 2018). We categorized a specimen a priori, as Penicillin Susceptible *S. aureus* (PSSA) if it was susceptible to Penicillin and Oxacillin,

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Methicillin Susceptible *S. aureus* (MSSA) if it was resistant to Penicillin and susceptible to Oxacillin. MRSA were resistant to Oxacillin. Multivariate logistic regression was used to determine the association between the presence of *S. aureus* isolates and the source of the clinical specimen (in vs. outpatient), age and gender. A P-value < 0.05 was considered statistically significant. A new case of *S. aureus* in the same patient was reported if the organism was isolated 21 or more days from the date of the first positive culture.

## RESULTS

### Prevalence

A total of 659 non-duplicate specimens with *S. aureus* isolates were included in the study. Initially we had 879 specimen collection forms however 220 were excluded due to incomplete data entry. PSSA was the most prevalent organism seen in all *S. aureus* cultures during the study period (60.85%, 401/659) while MRSA species were the least prevalent (0.61%, 4/659); 38.54% (254/659) of the isolates were MSSA. The annual prevalence of MRSA fluctuated between 0-1% during the five-year study period.

### Demographics

Of the isolates acquired, 62.37% (411) were from outpatients while 37.63% (248) were from inpatients. All MRSA isolates were cultured from inpatient samples. The average age of the study population varied from 25.73 to 34.58 years as shown in Figure 1 with an overall mean age of 29.66 years. Females constituted the majority of the study population-503/659 or 76.33%. MRSA was seen in male patients (3/4, 75%) unlike MSSA (100/254, 39.37%) and PSSA (52/401, 12.97%). *S. aureus* was largely isolated from wound swabs – (589/659, 89.38%). The prevalence of *S. aureus* bacteremia was 10.62% (70/659).

Similarly, MRSA reported was isolated in pus. The 2 pus specimens were obtained from an infected surgical wound and a diabetic foot infection. One MRSA was isolated from cerebrospinal fluid.

### Susceptibility patterns

All organisms isolated were sensitive to Vancomycin, Clindamycin and Linezolid. Oxacillin sensitive organisms accounted for 99.39% of the study population (Figure 2)(60.85%, 401/659), (70.71%, 466/659) and (83.31%, 549/659) of isolates were susceptible to Penicillin, Trimethoprim/sulfamethoxazole and Erythromycin respectively. Of the organisms isolated, 98.94, 98.03 and 99.70% respectively were sensitive to Gentamicin, Ciprofloxacin and Teicoplanin.

As a sub analysis we looked at the trend in penicillin resistance during the study duration. We focused on 3

commonly used penicillins in our setting- Amoxicillin, Amoxicillin-clavulanic acid and Penicillin G. The annual resistance to Penicillin G and Amoxicillin/clavulanic acid declined over this period, a trend that was statistically significant -Penicillin G (P value < 0.05, 95%CI 4.6376-28.3464) Amoxicillin-clavulanic acid (P value, < 0.001, 95% CI 37.50-85.83) (Figure 3). However, a significant increase in resistance to Amoxicillin was noted in the same period (P value < 0.001, 95%CI -50.4 to -29.44). Multivariate logistic regression confirmed no significant association between the probability of Penicillin resistance and site of collection (inpatient vs. outpatient), age or gender. (P=0.35, 0.98 and 0.07 respectively).

### Trends in mean antibiotic resistance

In 2014 *S. aureus* isolate was on average resistant to 3.02 antibiotics and by 2018 this decreased to 1.8 antibiotics. The number of MRSA isolated was too few to allow any further exploration of risk factors.

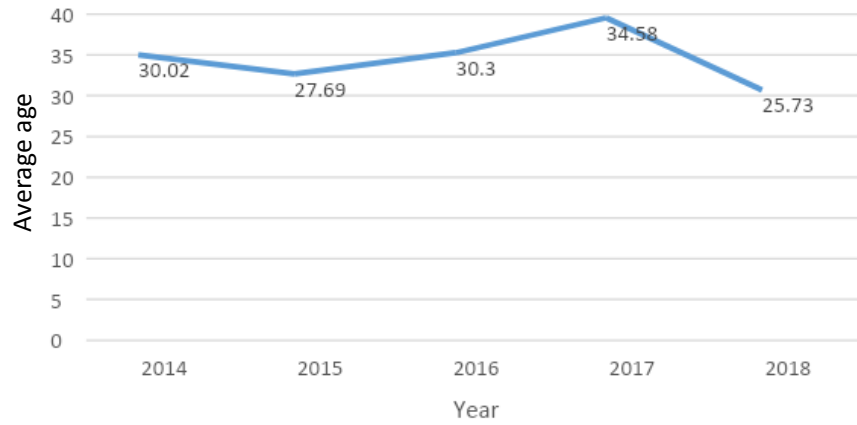
## DISCUSSION

In this retrospective review of a large number of *Staphylococcus* isolates, we demonstrate a low prevalence of MRSA and high proportion of *S. aureus* that were susceptible to penicillins. A similar prevalence has been reported in previous studies (Omuse et al., 2014). One study reviewed 731 specimens of *S. aureus* isolated in two private hospitals in Kenya found a low prevalence of MRSA (3.7%). This could be explained by low community transmission of MRSA (Aiken et al., 2014).

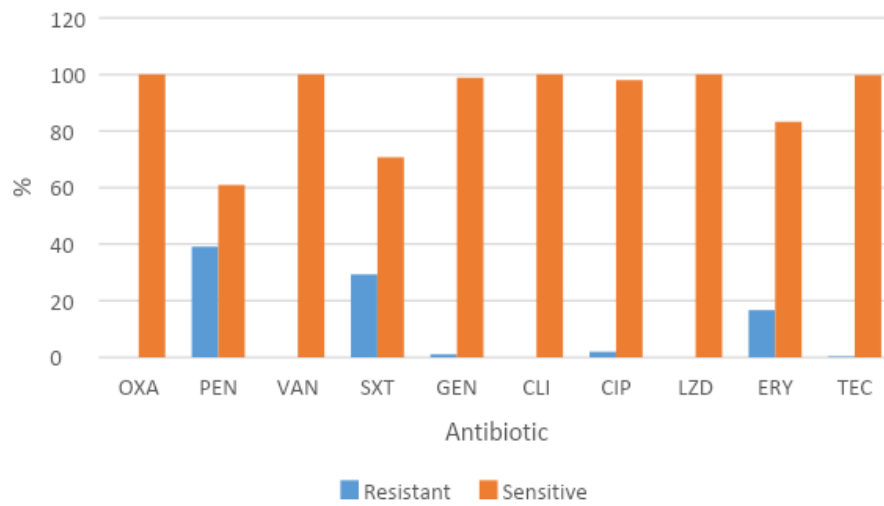
MSSA and PSSA were predominantly isolated in outpatients unlike MRSA which was more in inpatients. This has similarly been observed in Japan where MRSA was more prevalent in patients on admission (Nishijima et al., 1992). Even though this study was not designed to identify risk factors for MRSA acquisition, our site is not unique as it shares risk factors commonly associated with nosocomial acquisition of MRSA such as broad-spectrum antimicrobial therapy and admissions to intensive care units (Dilnessa and Bitew, 2016).

Majority of *S. aureus* isolates were from specimens cultured from patients between the ages of 0-19. In a study carried out in Australia (Agostino et al., 2017), MRSA infections were found to occur more often in younger patients (under 40). This transmission could be explained by the fact that younger people may have close skin-to-skin contact more often during participation in sports or when working.

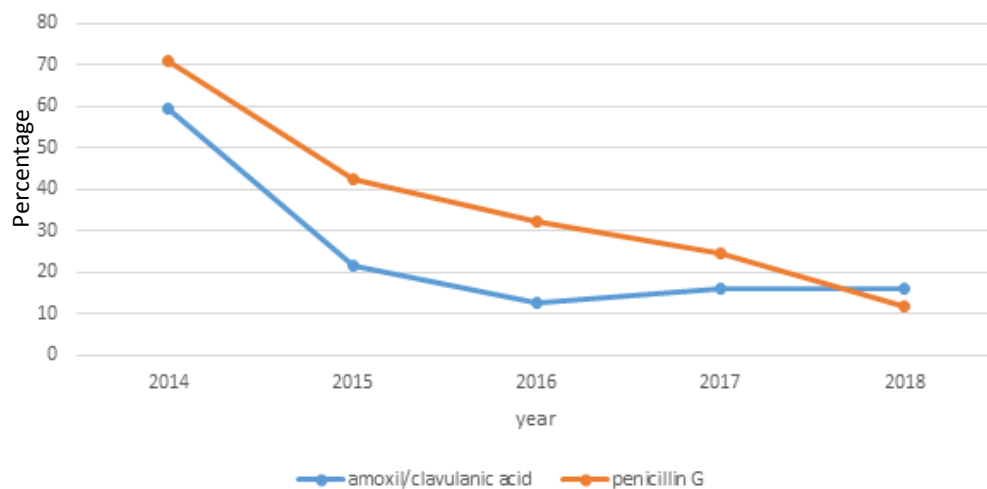
Most *S. aureus* isolates were from female patients. This difference could have arisen from the fact that female patients constituted the majority of the study population (76.33%) and this could further be explained by the



**Figure 1.** The average age of patients with *S. aureus* infections by year (2014 -2018).



**Figure 2.** *S. aureus* susceptibility to various antibiotics (2014-2018).



**Figure 3.** The trend of antibiotic resistance by *S. aureus* to Amoxicillin-Clavulanic acid and Penicillin G between 2014 and 2018.

differences in health seeking behavior across different genders (Thompson et al., 2016).

MSSA, PSSA and MRSA were all predominantly isolated from pus. This finding is similar to what was found by Dilnessa and Bitew (2016) in Ethiopia. MRSA was also isolated from a tracheal aspirate specimen and cerebrospinal fluid. The three clinical situations that predispose to the development of *S. aureus* meningitis are neurosurgical intervention, contiguous infection and *S. aureus* bacteremia. Until recently, most cases have been caused by Methicillin-susceptible *S. aureus* strains. There are now an increasing number of reports of severe infections attributed to methicillin-resistant strains of *S. aureus* (Pereira et al., 2015).

Methicillin Resistant *S. aureus* cultured in our setting was sensitive to Linezolid, Vancomycin, Tigecycline, Rifampicin, Fusidic acid, Teicoplanin and Tetracycline. These antibiotics have proved effective for treatment of MRSA infections in other parts of the world (Liu et al., 2011; Khalili et al., 2010). On the contrary, all the MRSA were resistant to Clindamycin which is considered an option in MRSA treatment in some parts of the United States of America (USA).

In our study half of MRSA isolates were susceptible to Trimethoprim-sulfamethoxazole (Pappas et al., 2009). Reported resistance of MRSA to Trimethoprim-sulfamethoxazole has been found to vary worldwide, in general being low in the industrialized world and higher in developing countries. Notably, in a sub analysis we found that resistance of *S. aureus* to Amoxicillin-Clavulanic acid and Penicillin G declined gradually over the past five years however, while resistance to Amoxicillin rose. This could be explained by the overuse of Amoxicillin which is more readily available and cheaper than amoxicillin-clavulanic acid and a decline in use of Penicillin G over the years (Ongarora et al., 2019). Furthermore, recent publications have reported a rise of Penicillin-Sensitive *S. aureus*. This trend has now been observed on several continents (Cheng et al., 2016). In the United States, this increase in the incidence of PSSA infections has also been mirrored by a decrease in Methicillin-resistant *S. aureus* infection rates (Butler-Laporte et al., 2018).

Overall antibiotic resistance in *S. aureus* in this site has declined in the past 5 years. One potential explanation for the trend of increasing *S. aureus* antibiotic susceptibility is a shift in antibiotic pressures. The decline in the use of narrow-spectrum beta-lactams such as Oxacillin and Penicillin G since 2000 and the inpatient use of first-generation cephalosporins since 2006 may select against hospital acquired MRSA in favor of PSSA (Kanjal et al., 2018). There are several limitations to our study. First, the small number of MRSA may preclude further analysis of risk factors and treatment options. Conclusions about MRSA as pertains to the choice of antibiotics for treatment of MRSA infections, in this setting must be interpreted carefully. Secondly, the testing for inducible beta-lactamase production was not performed. In

addition, in the absence of genotyping of specimens, phenotypic MRSA identification methods may not reflect an accurate picture. Lastly molecular testing of *S. aureus* was not employed in the study setting.

## Conclusion

The prevalence of MRSA was low. There were no strains that were resistant to Vancomycin, the drug of choice for MRSA. There has been a decline in resistance of *S. aureus* to Penicillin and Amoxicillin-clavulanic acid in the past five years.

## ABBREVIATIONS

**SCC mec**; Staphylococcal cassette chromosome *mec*, **MIC**; Minimum inhibitory concentration, **SSI**; Skin and soft tissue infection, **MSSA**; Methicillin-susceptible *Staphylococcus aureus*, **MRSA**; Methicillin-resistant *Staphylococcus aureus*, **CLSI**; Clinical and Laboratory Standards Institute, **VRSA**; Vancomycin Resistant *Staphylococcus aureus*, **OXA**; Oxacillin, **PEN**; Penicillin, **VAN**; Vancomycin, **SXT**; Trimethoprim – sulfamethoxazole, **GEN**; Gentamicin, **CLI**; Clindamycin, **CIP**; Ciprofloxacin, **LZD**; Linezolid, **ERY**; Erythromycin, **TEC**; Teicoplanin, **FA**; Fusidic Acid, **TET**; Tetracycline, **MXF**; Moxifloxacin, **RIF**; Rifampicin.

## AVAILABILITY OF DATA AND MATERIALS

The data and information supporting the conclusions of this article are available from the corresponding author on reasonable request.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol and procedure were approved by the ethics committee of the Mater hospital dated 9<sup>th</sup> November 2017. Our study does not include new clinical procedures, nor was it based on laboratory investigations, only on microbiological surveillance data. Our study did not involve any animals.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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