

Prevalence and antimicrobial susceptibility pattern of *Staphylococcus Aureus* isolated from clinical specimens at the Mater Hospital Nairobi, Kenya.

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Abstract

Staphylococcus aureus is a leading cause of hospital and community acquired infections globally. Surveillance of prevalence and antibiotic susceptibility patterns is important to ensure appropriate antibiotic prescription. The aim of this study was to determine the prevalence and susceptibility patterns of *Staphylococcus aureus* isolated from clinical specimens in a tertiary referral and training hospital.

A retrospective study was conducted at the Mater Misericordiae Hospital, Nairobi. It involved analyzing records of specimens analyzed between January 2014 and December 2018. Strains phenotypically resistant to <3 non β -lactam antimicrobial categories were defined as non-multidrug-resistant MRSA (nmMRSA) and strains that were resistant to ≥ 3 non- β -lactam antimicrobial groups were defined as multidrug-resistant MRSA (mMRSA). A specimen was categorized as Penicillin susceptible (PSSA) if susceptible to Penicillin and Oxacillin, MSSA if resistant to Penicillin and susceptible to Oxacillin, and MRSA if resistant to Oxacillin and Penicillin.

Results

PSSA accounted for 60.85%, (401/659) of *S. aureus* cultured. The prevalence of MSSA was 38.54% (254/659). MRSA was the least prevalent. (0.61%, 4/659).

No multi-drug Methicillin resistant *S. aureus* were isolated in the five-year period. PSSA accounted for 60.85% (401/659) of all *S. aureus* isolates. 100% of MRSA isolated was sensitive to Vancomycin, Linezolid, Tigecycline, Tetracycline and Rifampicin. MRSA was resistant to Clindamycin and Erythromycin.

Conclusion;

The prevalence of MRSA was low compared to previous studies. All strains isolated were non-multidrug resistant. There was no resistance to Vancomycin detected. Penicillins are still a viable option in the treatment of majority of *S. aureus* infections in our setting.

Background

According to (Daniel J. Diekema et al., 2019), *Staphylococcus aureus* (*S. 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. Furthermore, they highlighted that antibiotic resistance is a major problem in the treatment of infections by *S. aureus*. The emergence of penicillinase-producing *S. aureus* strains occurred shortly after the introduction of penicillin for clinical use, and by the 1970s majority of *S. aureus* infections were penicillin resistant. Methicillin resistance among *S. aureus* was reported in the early 1960s, after the introduction of methicillin following the acquisition of the *mecA*-containing *Staphylococcal* cassette chromosome *mec*(SCC*mec*) (Kanjilal et al., 2018). Research has shown that the emergence of Methicillin Resistant *S. aureus* (MRSA) has complicated treatment of *Staphylococcus aureus* infections (Daniel J. Diekema et al.,

2019),. In addition infections caused by MRSA are associated with significant morbidity, mortality and cost. MRSA strains are not only resistant to nearly all beta-lactams, but many have developed resistance to multiple other classes of antimicrobials. (D. J. 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. According to (Limbago et al., 2014) 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 among MRSA. In a study carried out in the United States, it was found that the prevalence of MRSA blood infections in the United States and Europe has dropped in recent years (Sutter et al., 2016). Similarly (Abubakar & Sulaiman, 2018), found MRSA infection rate in Asia is also declining. 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) and (Abubakar & Sulaiman, 2018) .According to (Kanjilal et al., 2018) the changing trajectory of MRSA infection in developed countries has been attributed to implementation of control interventions. More recent data by (Cheng et al., 2016) further suggests that penicillin susceptibility may be in a period of renaissance.

In Africa, MRSA prevalence intra- country and intercountry has been reported to be heterogeneous. According to (Wangai et al., 2019) antimicrobial resistance data from Kenya has been variable and inconsistent due to lack of effective and systematic routine surveillance systems .

The objective of our study was to establish the prevalence of *S. aureus* and MRSA, 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. The knowledge acquired will be used to guide the choice of antibiotic therapy and serve as a baseline for measuring the impact of interventions.

Methods

This study was carried out in the microbiological laboratory in Mater hospital, a tertiary referral hospital and training center in Nairobi, Kenya. It serves a large and diverse low and middle income population comprising of patients of African and Asian descent and few Caucasians. The Mater hospital microbiological laboratory is ISO 2012 certified. This study was well powered due to the large number of records reviewed. One study by (Gitau et al., 2018) analyzed 944 specimens of *S.aureus* while (Omuse et al., 2015) looked at 731 non duplicate specimens *S.aureus* so we felt our study population of 659 non duplicate *S.aureus* specimens was sufficient.

Data and sample collection

This was a retrospective study based on electronic laboratory records from the Mater microbiological laboratory. The following demographic information was extracted from the hospital administrative database about each patient- in vs. outpatient status, age and gender, and antimicrobial susceptibility profiles of *S. aureus*. Data from 2014 to 2018 was retrieved from the hospital medical records and for 2018 from Vitek 2 (bioMérieux, 2018) antibiotic susceptibility system imported to (LIFELINE, 2014) database.

Standard operating procedures were followed in collection of samples in the five-year period.

Clinical samples from various anatomical sites of study participants were cultured on blood agar and mannitol salt agar and identified to be *S. aureus* by using catalase and coagulase tests. Tests were performed *in vitro*, and measured the growth response of an isolated organism to a particular drug or drugs. These tests were performed under standardized conditions so that the results were reproducible. The raw data was either in the form of a **zone size** or **MIC**.

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).

After incubation, the diameters of the complete growth inhibition zones around each disk were measured. Pre-specified breakpoints were used to interpret the zone sizes and classify them as susceptible(S), intermediate (I), or resistant(R). 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 was determined using Oxacillin. This is similar to a study by (Udobi et al., 2013) where analysis was done according to the NCCLS (2002) guidelines using oxacillin in agar screen test. Isolates from a solution adjusted to 0.5 McFarland standard were spot inoculated onto Mueller Hinton agar supplemented with 6 micrograms/mL oxacillin and 4% sodium chloride. The plates were incubated at 35°C for 24 hours. The isolates that survived showing more than one colony were considered Methicillin resistant. Approval for this retrospective study was obtained from the Ethics committee of the Mater hospital. All patient information was anonymized preceding the analysis.

Data Analysis

The data collected was analyzed using (IBM-SPSS, 2018). We categorized a specimen a priori, as Penicillin Susceptible *Staphylococci aureus*(PSSA) if it was susceptible to Penicillin and Oxacillin, Methicillin Susceptible *Staphylococci aureus* (MSSA) if it was resistant to Penicillin and susceptible to Oxacillin, and MRSA if it was resistant to Oxacillin and Penicillin. (Kanjilal et al., 2018) The findings were presented in tables, graphs and charts. Bivariate analysis was done by use of Chi square to assess for association between categorical variables such as susceptibility of antibiotics and infections location (Gitau et al., 2018). 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.

Results

Prevalence;

A total of 659 specimens with *S. aureus* isolates were included in the study. Initially we had 879 specimen collection forms however 220 were eliminated due to incomplete data.

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

62.37 % (411) of all specimens were collected from outpatients as compared to 37.63 % (248) from admitted patients. All MRSA isolates were cultured from specimens from inpatients (general medical wards (50%, 2/4) as compared to the intensive care unit (25%, 1/4))

The average age of all patients with *S. aureus* infections (N=659) was 29.66

MRSA cultured was predominantly from specimens obtained from patients between the age of 0-19(2/4, 50%).

There were no distinct differences in age between patients with MSSA and MRSA. Patients with MRSA infections were younger than patients with either PSSA (median age 27.77 vs 30.17 years) or MSSA (median age 27.77 years vs. 28 years) (p-value =0.875) but this age difference was not found to be significant. (P-value =0.89)

Females constituted the majority of the study population.503/659, (76.33%). MRSA was seen more in male patients (3/4,75%) unlike MSSA (100/254, 39.37%) and PSSA (52/401,12.97%).

S. aureus was largely detected in pus – (589/659, 89.38%). Majority of the pus specimens were obtained from wounds. The prevalence of *S. aureus* bacteremia was 10.62% (70/659)

Similarly, MRSA was most prevalent in pus (2/4, 50%).The 2 pus specimens were obtained from an infected surgical wound and a diabetic foot infection. A unique case of MRSA was cultured from cerebrospinal fluid.

Susceptibility patterns

Antibiotic	Resistant N(%)	Sensitive N(%)
Oxacillin(6mcg/ml)	4(0.61)	655(99.39)
Penicillin G (10u)	258(39.15)	401(60.85)
Vancomycin**	0	659(100)
Sxt*(1.25/23.75mcg)	193 (29.29)	466 (70.71)
Gentamycin(10mcg)	7 (1.06)	652 (98.94)
Clindamycin (30mcg)	0	659(100)
Ciprofloxacin(5mcg)	13 (1.97)	646 (98.03)
Linezolid	0	659 (100)
Erythromycin(15mcg)	110 (16.69)	549 (83.31)
Teicoplanin	2(0.30)	657 (99.70)

*Trimethoprim-sulfamethoxazole

**2mcg-16mcg/ml

As a sub analysis we looked at the trend in penicillin resistance during the study duration. The annual resistance to Penicillin G and Amoxicillin/clavulanic acid declined in the study period and was found to be statistically significant-Penicillin G (P value=0.018, 95%CI 4.6376-28.3464) Amoxicillin-clavulanic acid (P value=0.002,95% CI-37.50-85.83). However, a significant increase in resistance to Amoxicillin was noted in the same period (P value=0.000, 95%CI -50.4 to -29.44)

Multivariate logistic regression confirmed no significant association between the probability of Penicillin resistance and site of collection (in vs. outpatient), age or gender. (P=0.35,0.98 and 0.07 respectively)

MRSA

MRSA accounted for (4/659),0.61%of isolates. No mMrsa were isolated

MRSA isolated was 100% resistant to Quinolones, Clindamycin and Erythromycin.

Trends in mean antibiotic resistance

In 2014 a *S. aureus* isolate was on average resistant to 3.02 antibiotics and by 2018 this decreased to 1.8 antibiotics.

Discussion

The current study demonstrates that there has been a high prevalence of PSSA (60.85%) and a low prevalence of MRSA (0.61%) and MSSA (38.54%). A similar prevalence has been reported in previous studies by (Omuse et al., 2015). This could be explained by low community transmission of MRSA and MSSA (Aiken et al., 2014).

MSSA and PSSA were predominantly isolated in outpatients unlike MRSA which was more in admitted patients. This has similarly been observed by (Nishijima et al., 1992) in a study in Japan where MRSA was more prevalent in admitted patients. Even though this study was not designed to identify risk factors for MRSA acquisition, risk factors that have previously been associated with acquisition of MRSA in hospitals such as broad-spectrum antimicrobial therapy, admission to an intensive care unit and proximity to other patients with MRSA could play a major role in our study site (Dilnessa & Bitew, 2016).

Majority of *S. aureus* isolates were from specimens cultured from patients between the ages of 0-19. However, there were no significant differences in age between patients with MRSA and MSSA. 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 younger people may have close skin-to-skin contact more often during participation in sports or when working.

There were statistically significant differences in gender between patients who grew MRSA and PSSA. Similarly, there were significant differences in gender between patients with MSSA and PSSA however there were no significant differences in gender between patients whose cultures were positive for MRSA and MSSA. Most *S. aureus* isolates were from female patients. This difference could have arisen from the fact that female patients constituted majority of the study population (76.33%) and this could further be explained by the differences in health seeking behavior across different genders (Thompson et al., 2016).

MRSA infections were highest in male patients unlike MSSA and PSSA. Several studies have found the isolation, carriage and infection of MRSA to be higher in males (Humphreys et al., 2015). This could be because hand-hygiene behavior varies according to gender. Males are less compliant, which in turn may predispose them to higher colonization and infection rates.

MSSA, PSSA and MRSA were all predominantly isolated from pus. This finding is similar to what was found by Dilnessa et al in Ethiopia (Dilnessa & Bitew, 2016). 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 isolates cultured in our setting were resistant to Clindamycin which is considered an option in MRSA treatment in some parts of the United States of America(USA).

Notably, in a sub analysis we found that resistance of MSSA and PSSA to Amoxicillin-Clavulanic acid and Penicillin G declined gradually over the past five years however, 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. Furthermore, recent publications have reported a rise of Penicillin-Sensitive *Staphylococcus aureus*. This trend has now been observed on several continents _and has been shown to involve bloodstream infections and other serious infections (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) .

In our study 50%of MRSA were susceptible to trimethoprim-sulfamethoxazole. (Pappa et al., 2009) reported resistance of MRSA to trimethoprim-sulfamethoxazole to vary worldwide, in general being low in the industrialized world and higher in developing countries.

Overall the annual antibiotic *S. aureus* resistance 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 (Kanjilal et al., 2018).

There are several limitations to our study. First, the testing for inducible beta-lactamase production was not performed, raising the possibility that specimens reported as penicillin susceptible from this time period were in fact penicillin resistant. In addition, we did not collect information on a number of vital aspects such as history of antimicrobial use and duration of hospital admission. Furthermore, in the absence of genotyping of specimens, phenotypic MRSA identification methods may not reflect an accurate picture.

Conclusion

The prevalence of MRSA was low when compared with the prevalence rates obtained in previous studies conducted in Kenya. MRSA strains were non-multidrug-resistant and 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. Good infection control practices and prudent antibiotic use can help to further reduce the burden of MRSA in our setting.

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 *Staphylococci aureus*

CLSI-Clinical and Laboratory Standard Institute

VRSA- Vancomycin Resistant *Staphylococci 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

Declarations

Authors' contributions

CO participated in the study design, literature search, data collection, data analysis, manuscript writing and is the corresponding author, CM, FCFO, LAO and EO participated in the study design, writing the manuscript, interpretation of data, supervision of the microbiological diagnosis and served as scientific advisors. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests

Availability of data and materials

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

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study protocol and procedure were approved by the ethics committee of the Mater hospital dated 9th 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.

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Table

Demographic and microbiologic characteristics of patients' and *S. aureus* subtype

Subtype	No. of	Mean patient	%				
	isolates	age in	female	blood	Lung	SSI	Other
		years(s.d)	patients				
All S.aureus	897	29.73 (21.38)	57.2	7.85%	3.07%	73.27%	15.81%
MRSA	4	31.97 (37.06)	25	0	25%	50%	25%
MSSA	220	28.32 (22.01)	52.73	5.91%	1.36%	83.64%	9.09%
PSSA	401	32.84(20.93)	60.05	5.79%	3.27%	71.54%	19.4%
P-value							

MRSA vs MSSA		0.75	0.705	-	0.003	0.003	0.003
MRSA vs PSSA		0.947	<0.0001	-	<0.0001	<0.0001	<0.0001
MSSA vs PSSA		0.012	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Tests of difference are two-sided and comprised *t* tests for mean age, chi-squared tests for sex and site of infection.

Figures

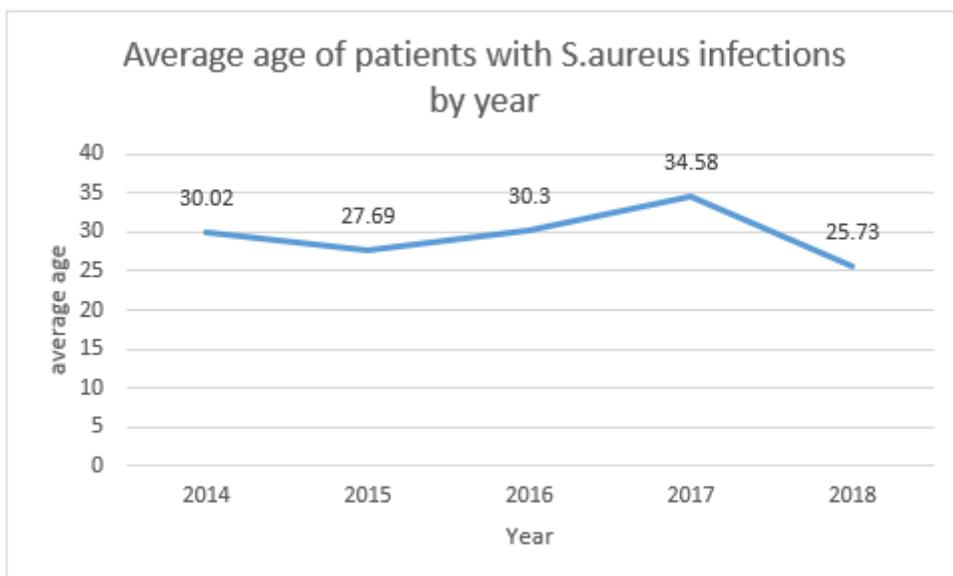


Figure 1

The average age of all patients with *S. aureus* infections (N=659) was 29.66

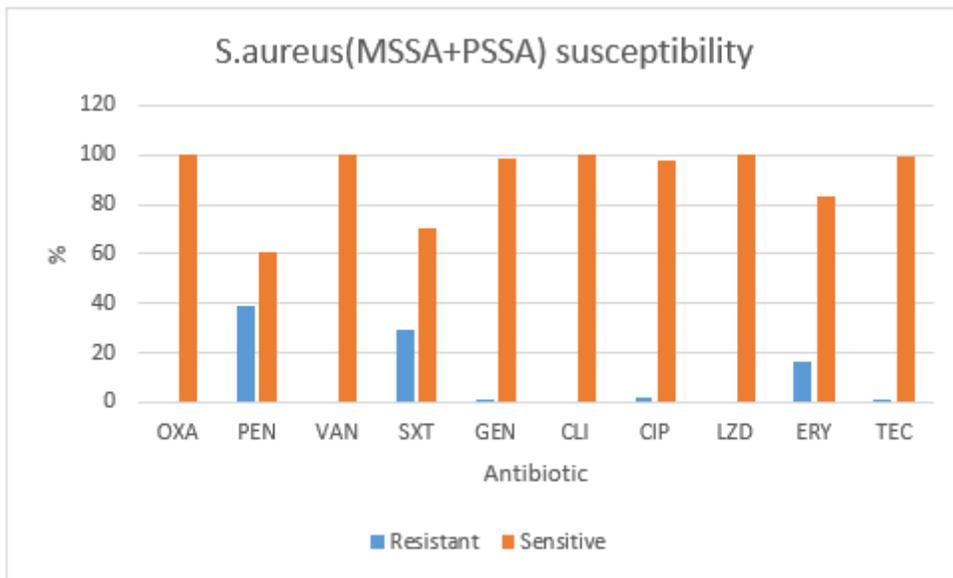


Figure 2

S. aureus susceptibility

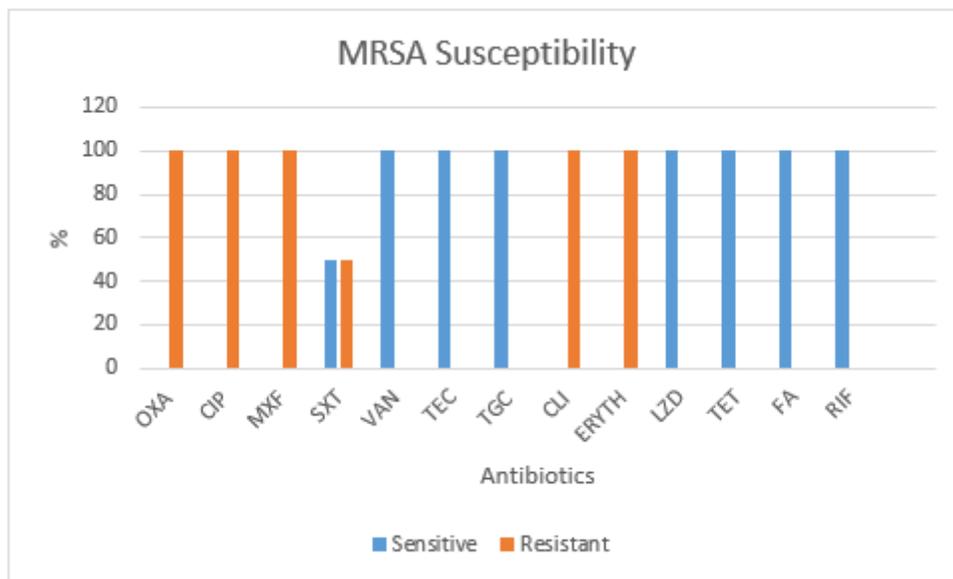


Figure 3

MRSA susceptibility